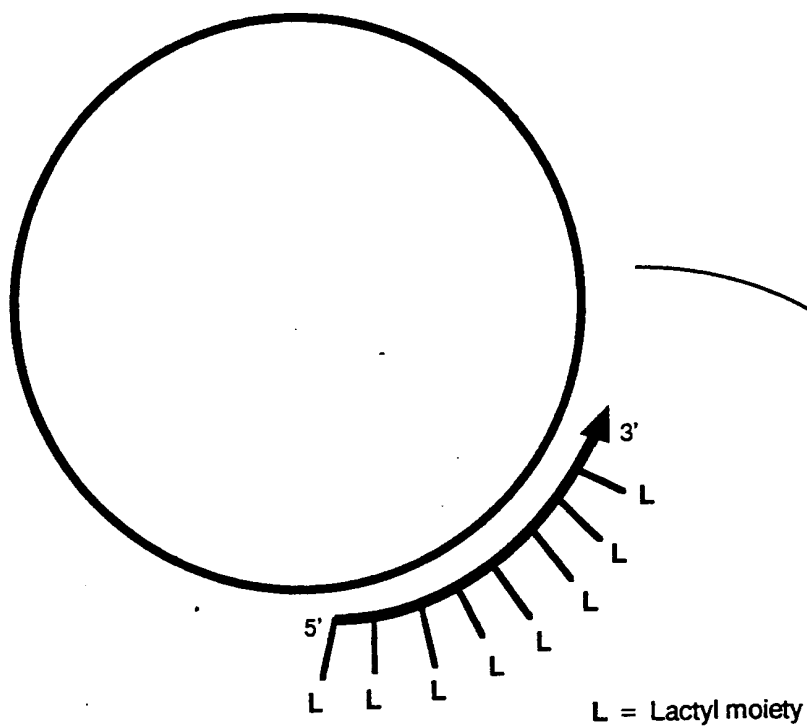
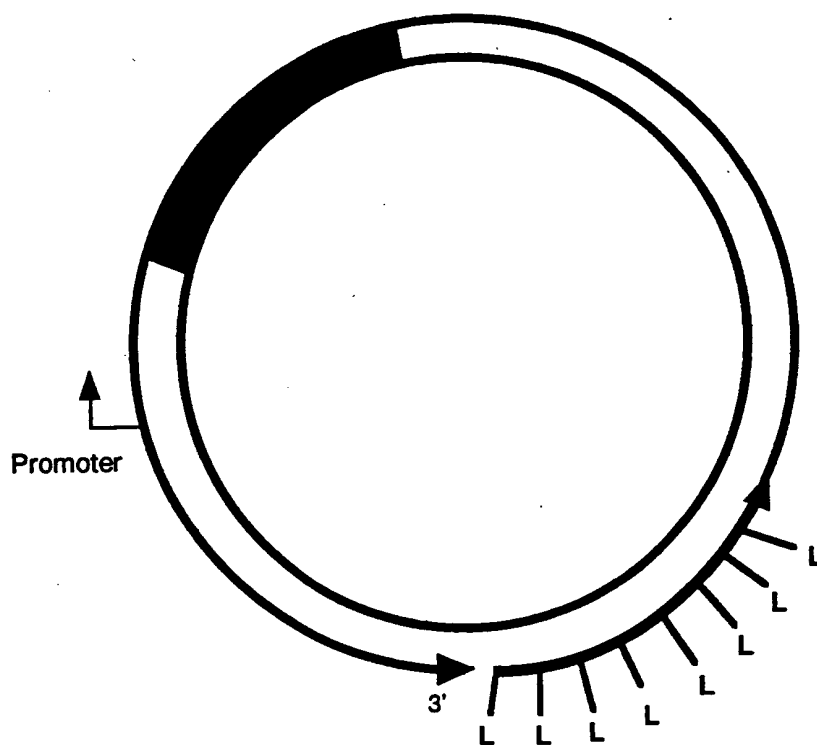


(a)



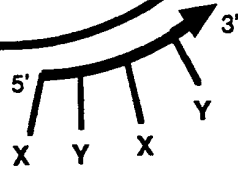
(b)

**Figure 1****Attachment of Ligands Through Primer Region**

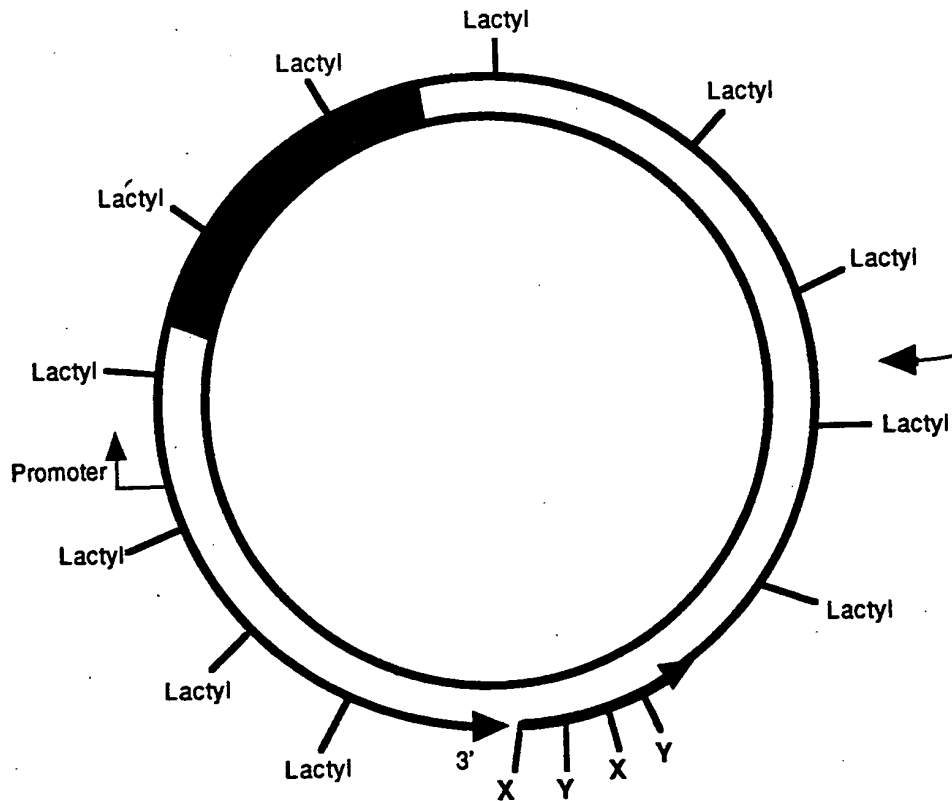
(a)

X = Nuclear Localisation Signal

Y = fusogenic peptide

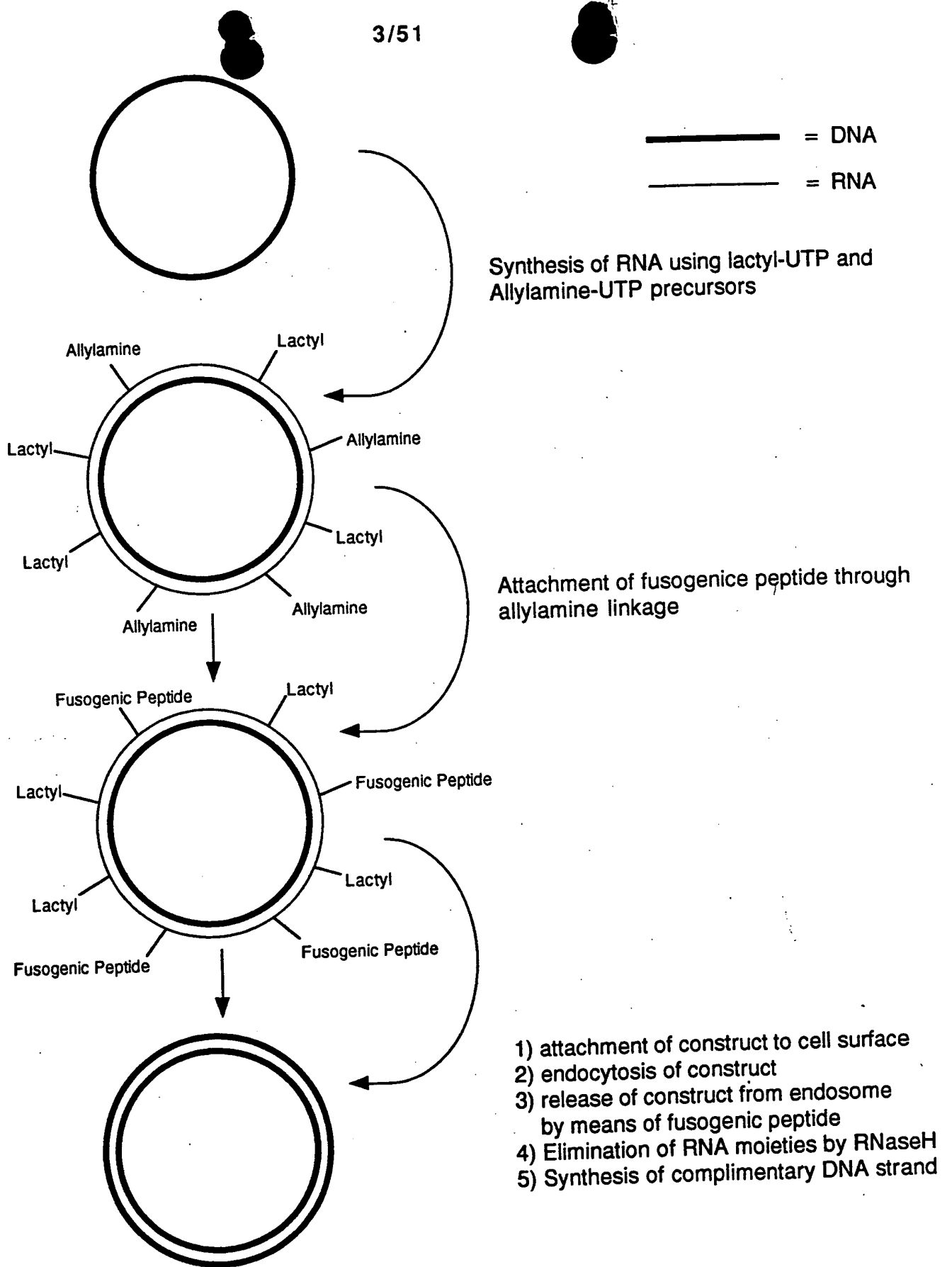


(b)

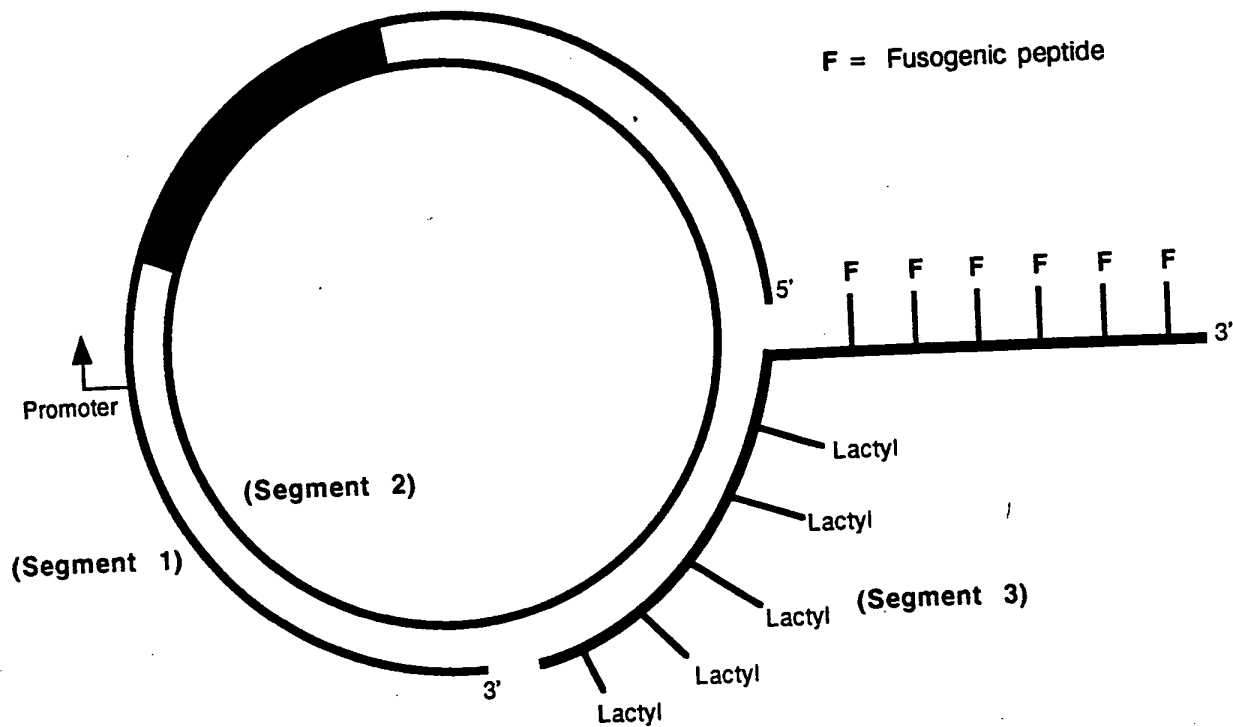


**Figure 2**  
Attachment of Ligands by Incorporation of  
Modified Nucleotide Precursors

08978532, 112597

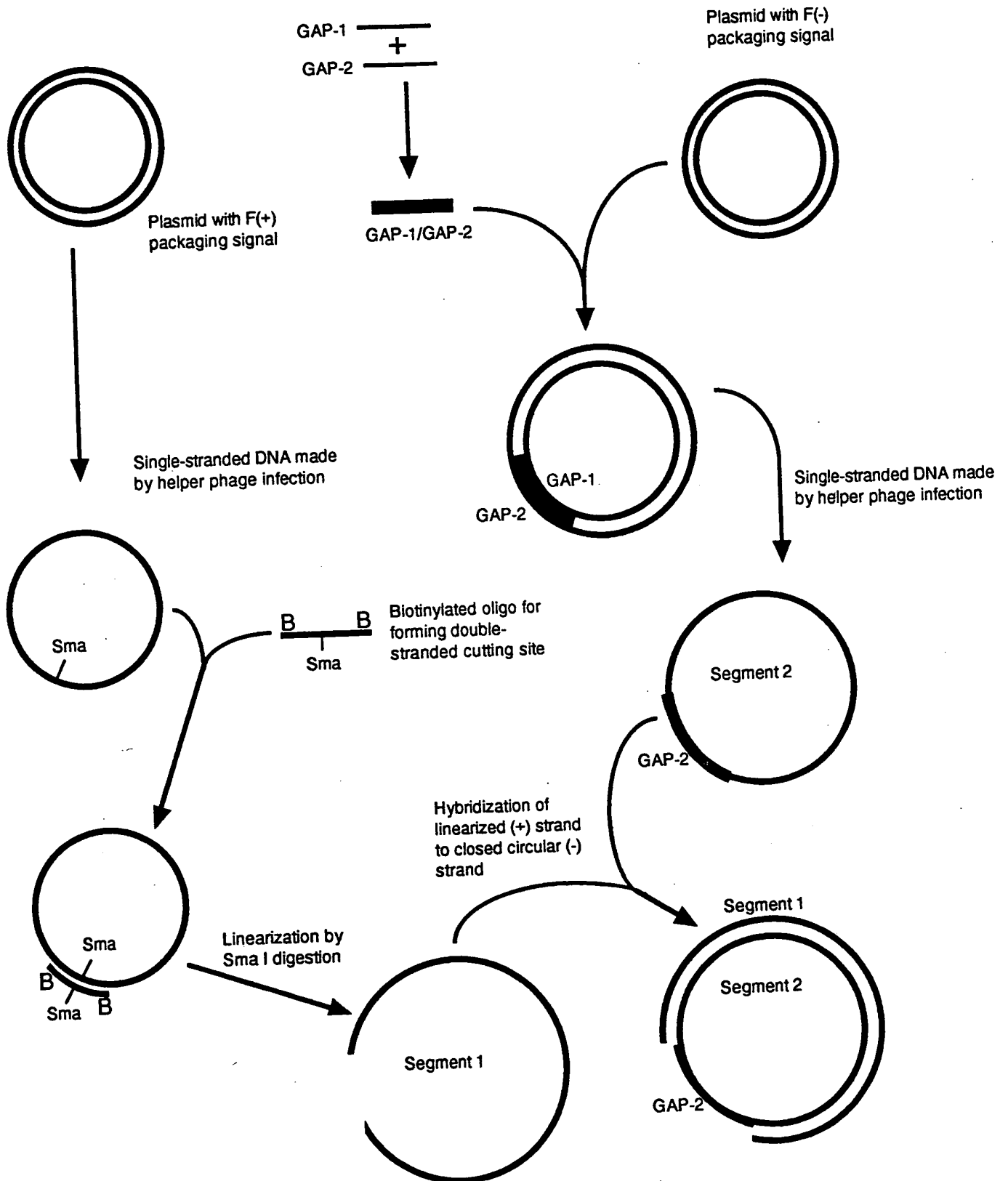


**Figure 3**  
Incorporation of Ligands through Modified Ribonucleotides



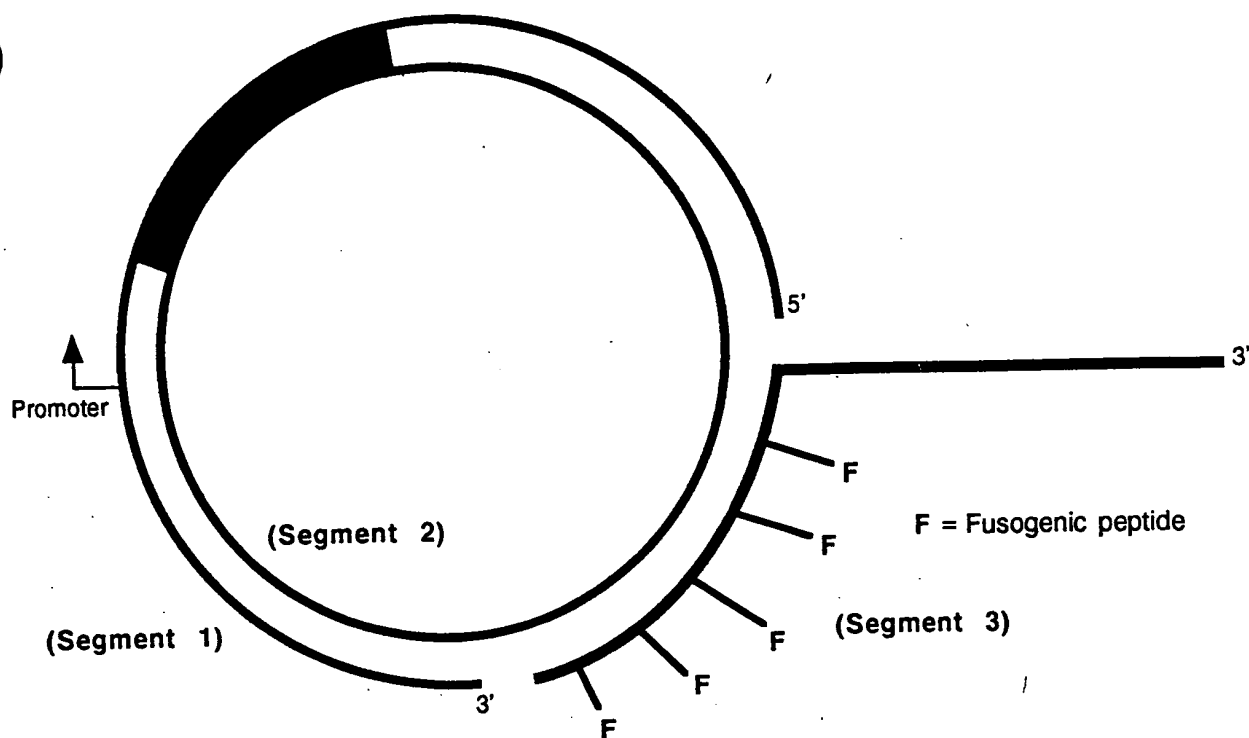
**Figure 4**

Attachment of Ligands through a 3' tail

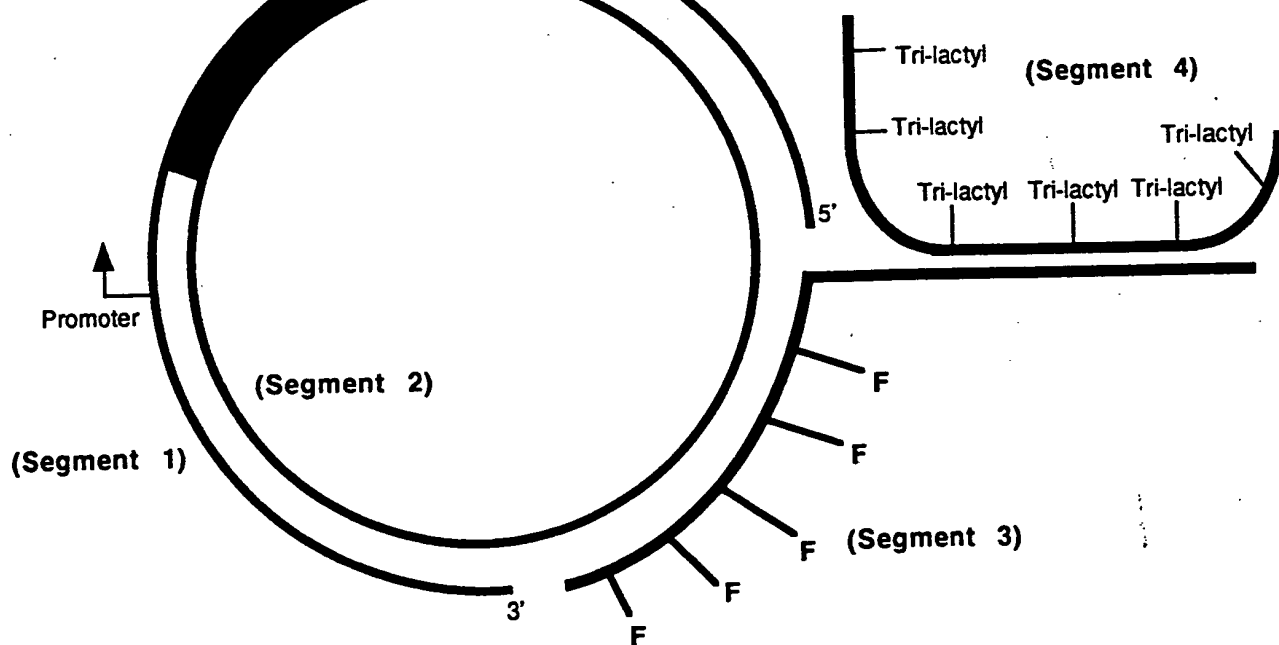


**Figure 5**  
Preparation of Gapped Circle

(a)



(b)

**Figure 6**

Attachment of Ligands through hybridization to a 3' tail

08978632.112597

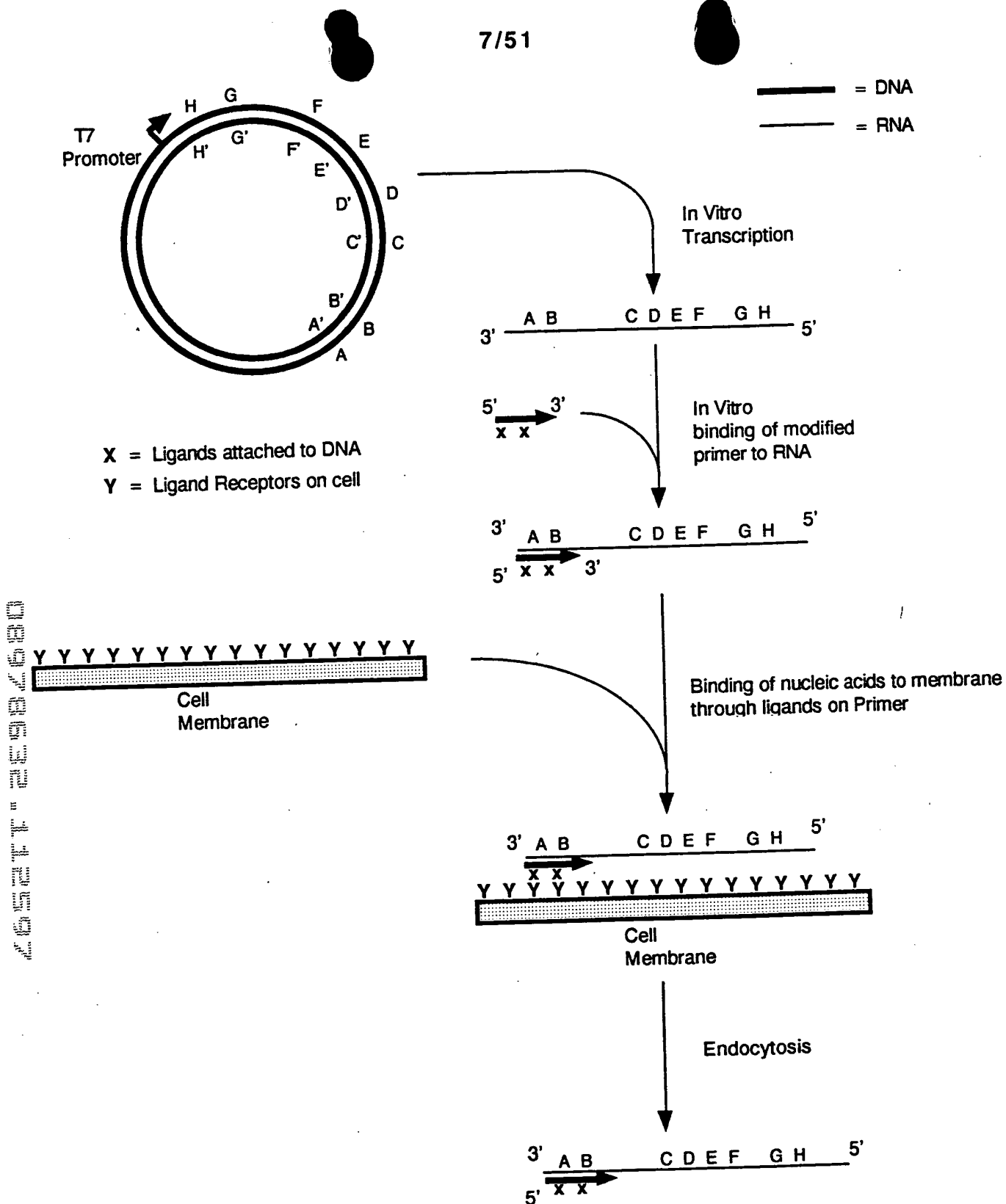
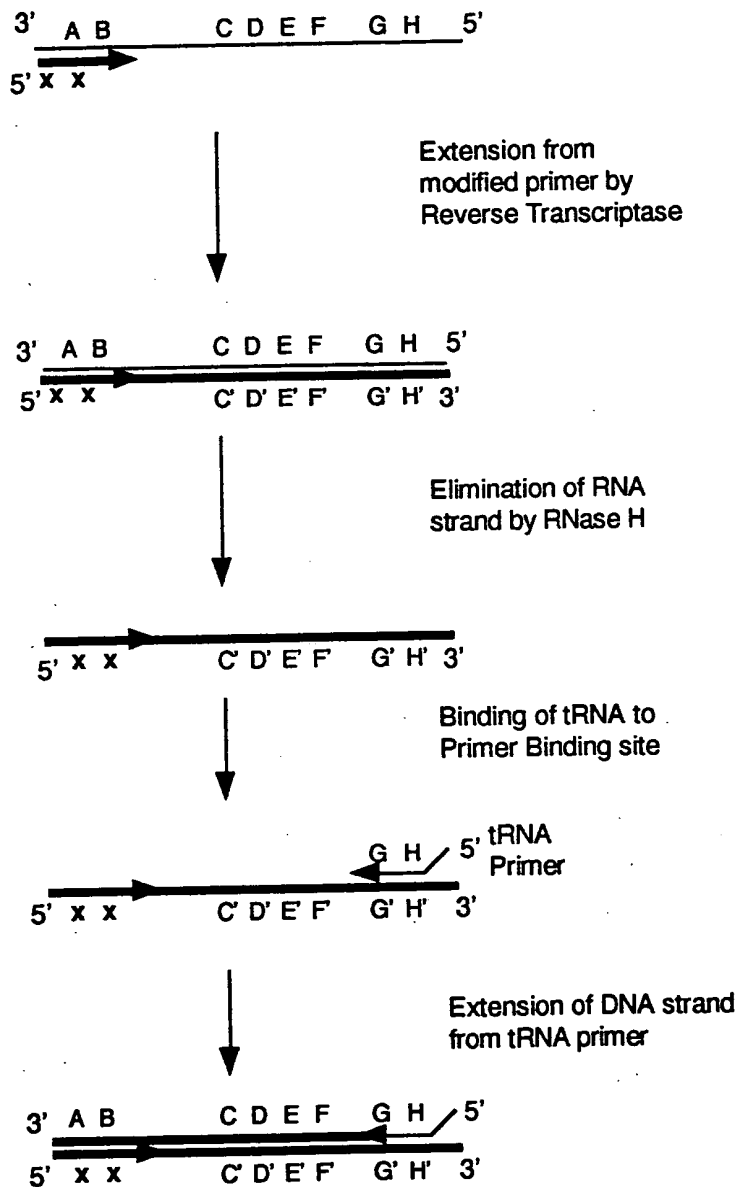


Figure 7

RNA with Ligands on Primer

(Continued in Figure 8)

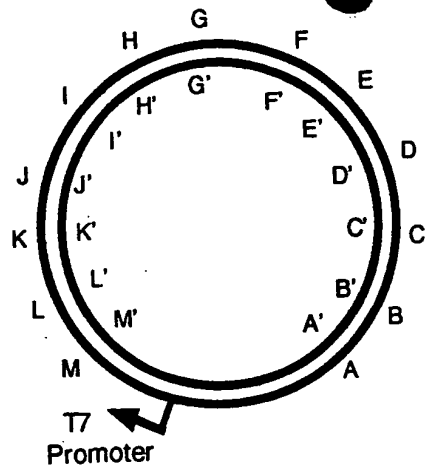
Continued from Figure 7

**Figure 8**

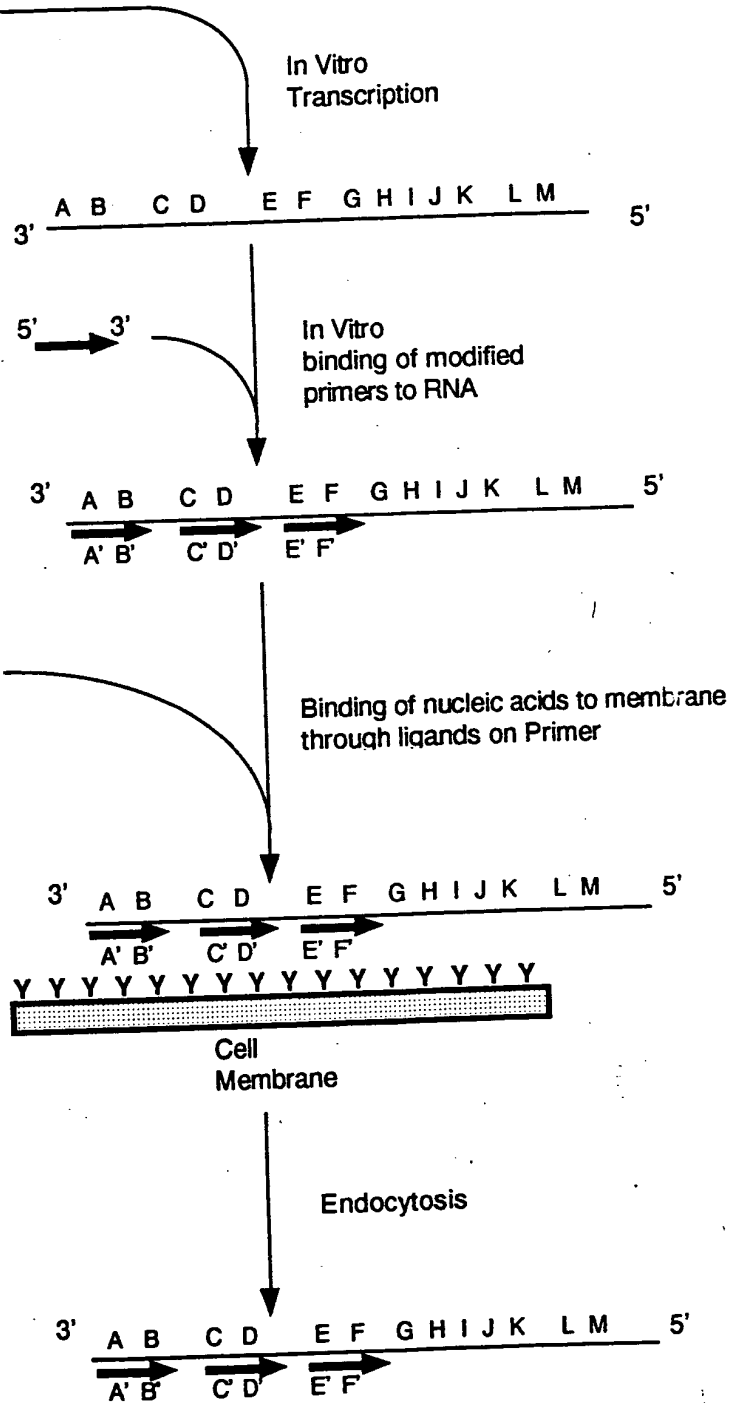
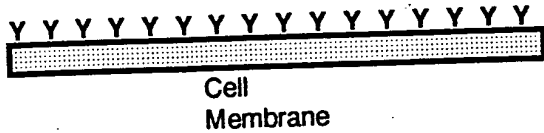
RNA with Ligands on Primer (Continued)



— = DNA  
 — = RNA



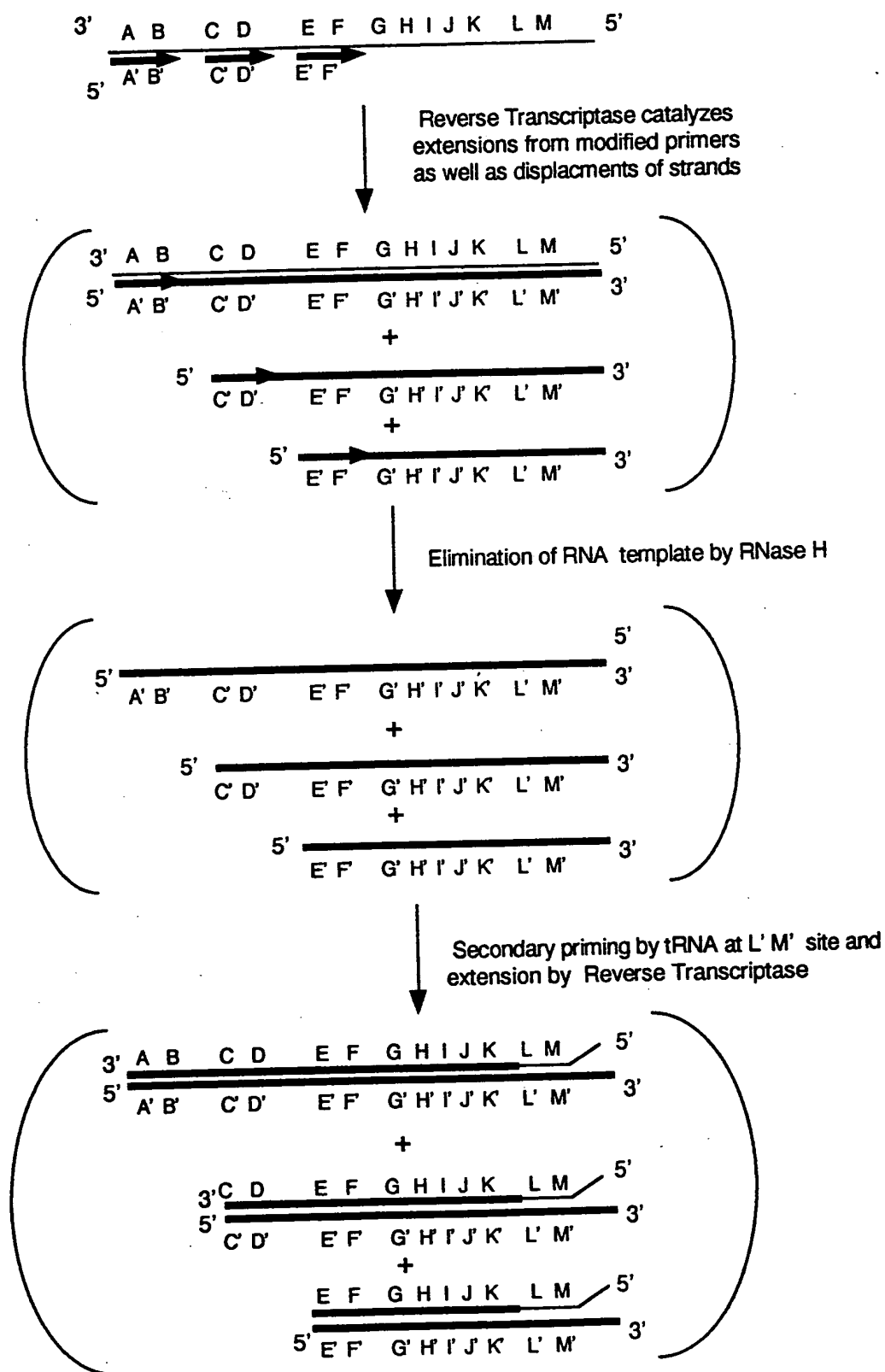
Y = Ligand Receptors on cell



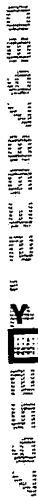
(Continued in Figure 10)

**Figure 9**  
 RNA with Ligands on Multiple Primers

Continued from Figure 9

**Figure 10**

RNA with Ligands on Multiple Primers (Continued)



(Continued in Figure 12)

**Figure 11**  
**Single-stranded DNA with attached Ligands**

Continued from Figure 11

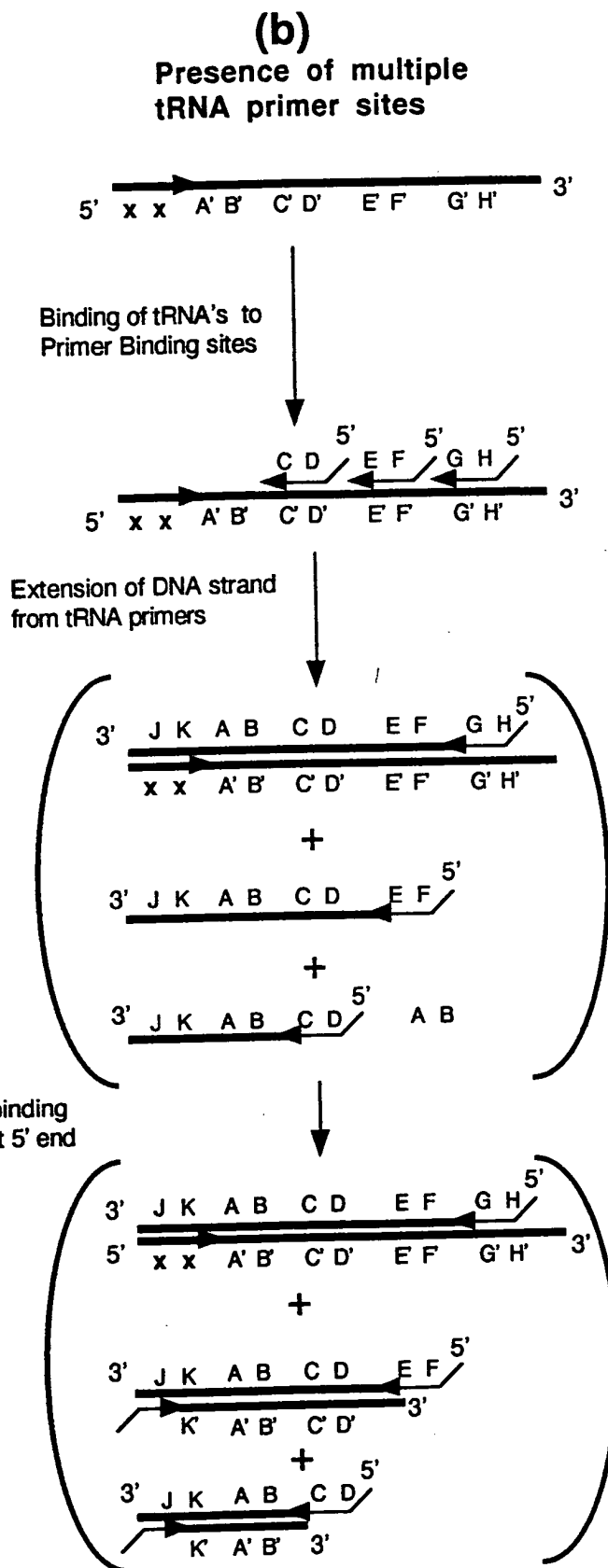
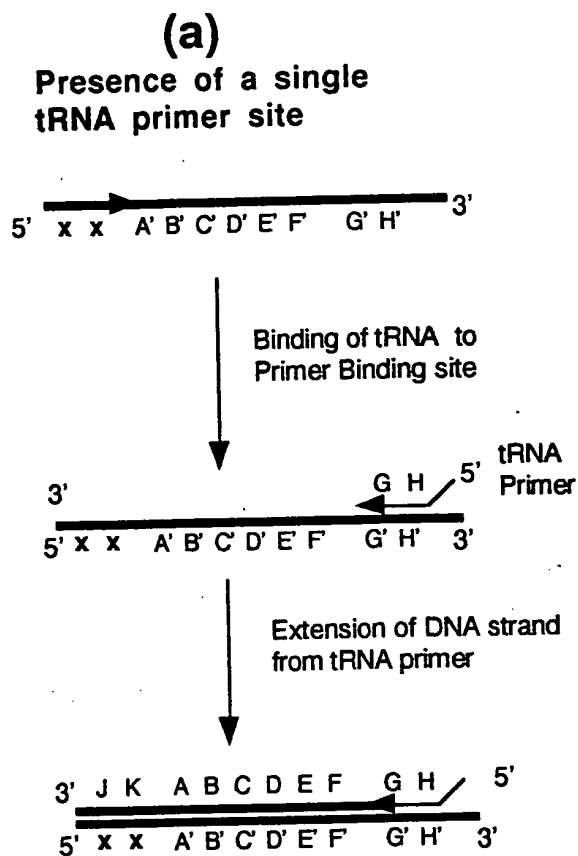
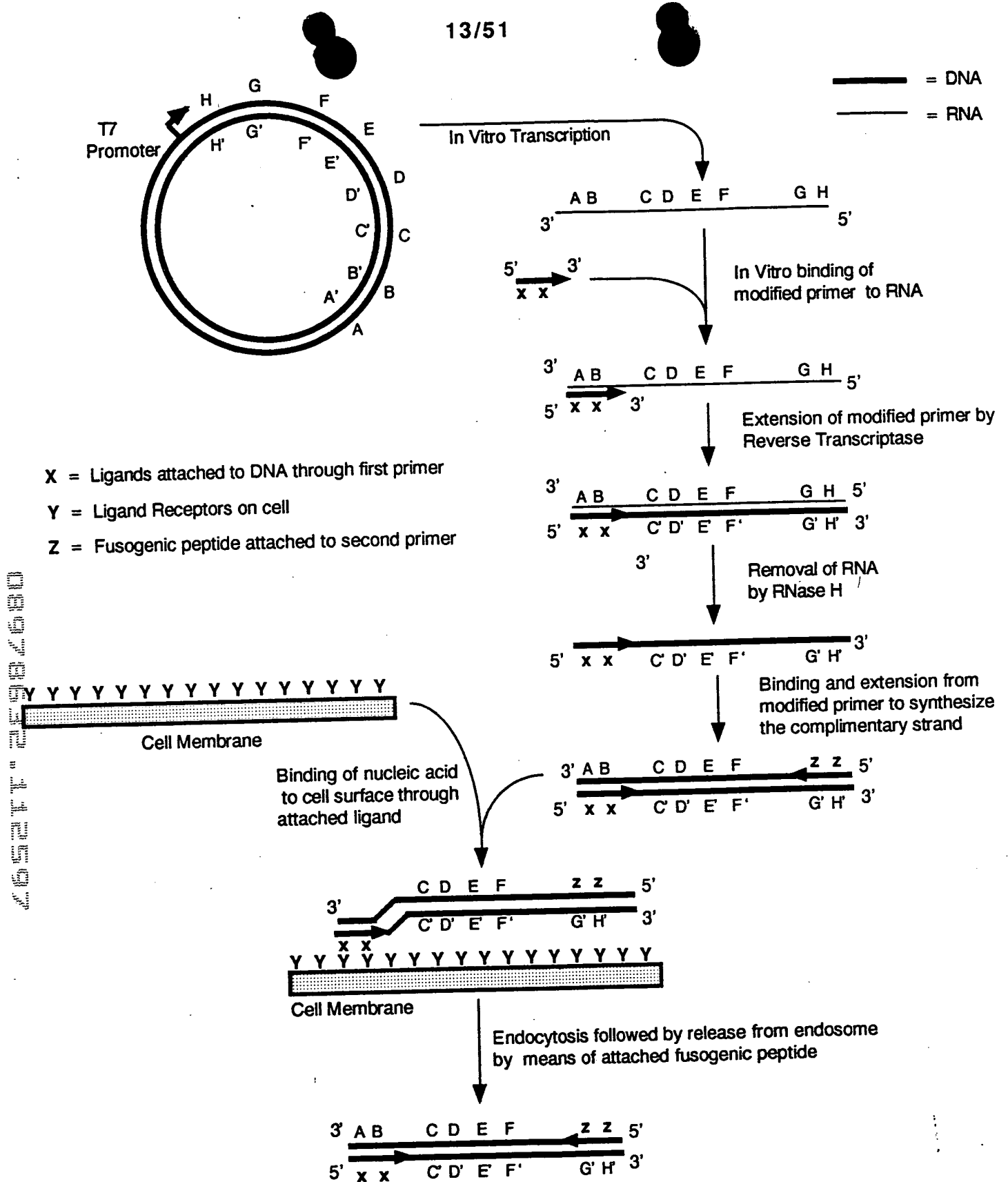


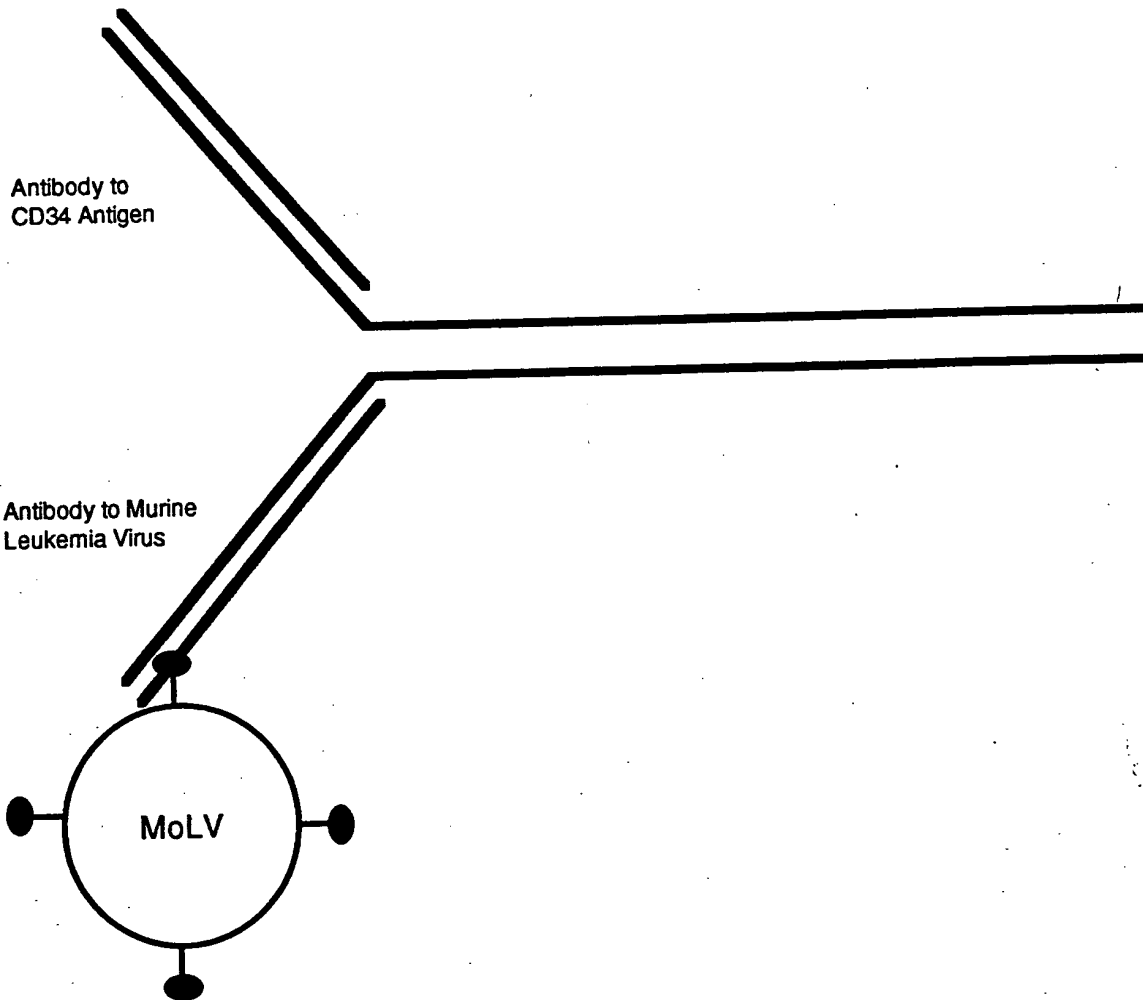
Figure 12

Single-stranded DNA with attached Ligands (continued)



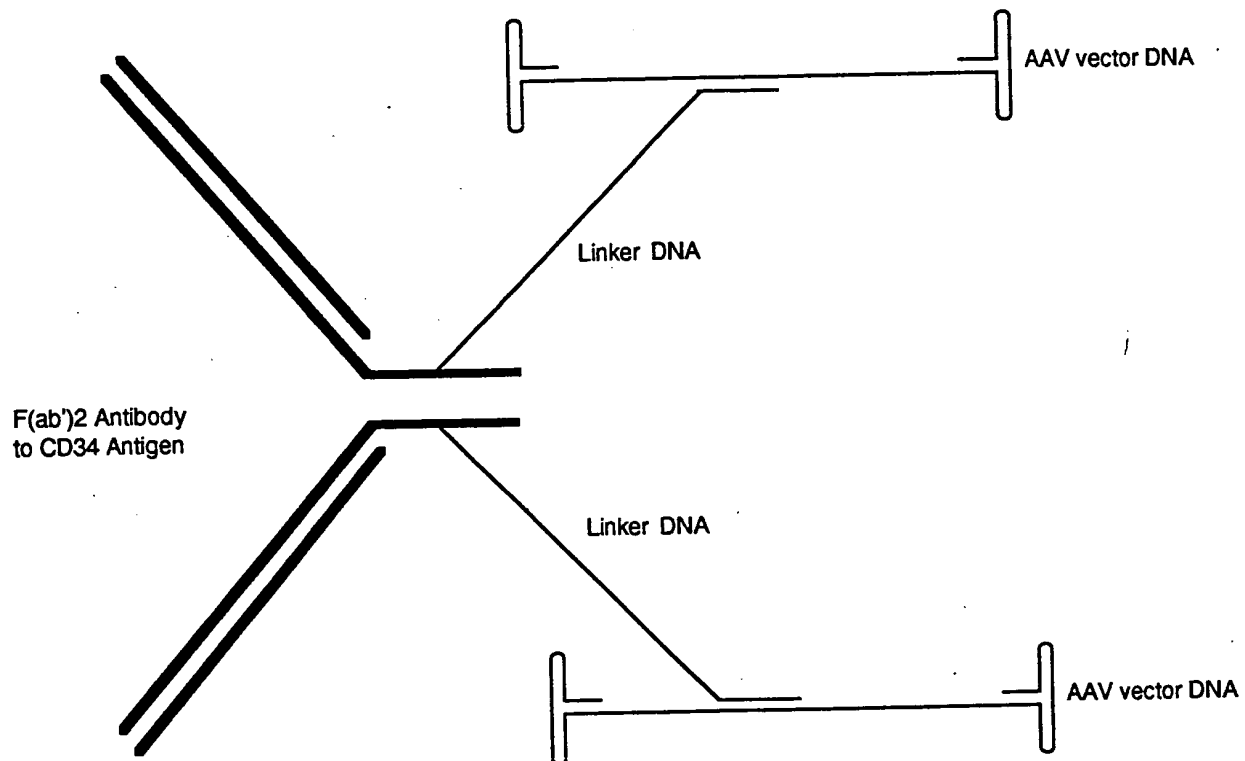
**Figure 13**

Linear Double-stranded DNA with attached Moieties on each strand



**Figure 14**

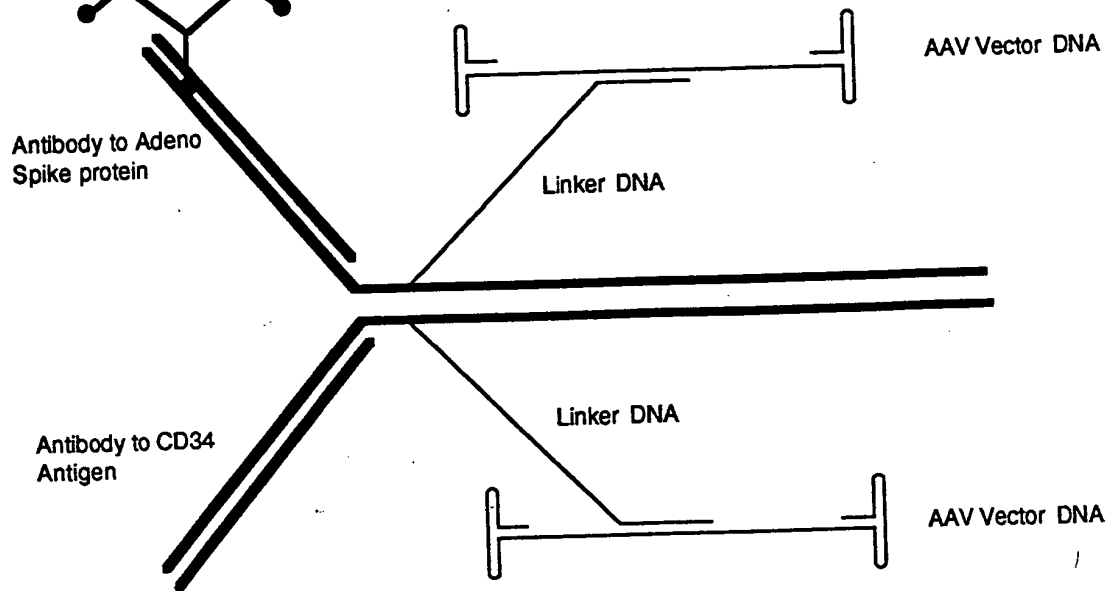
Enhanced Delivery of Retroviral Vector  
to Haematopoietic Stem Cell



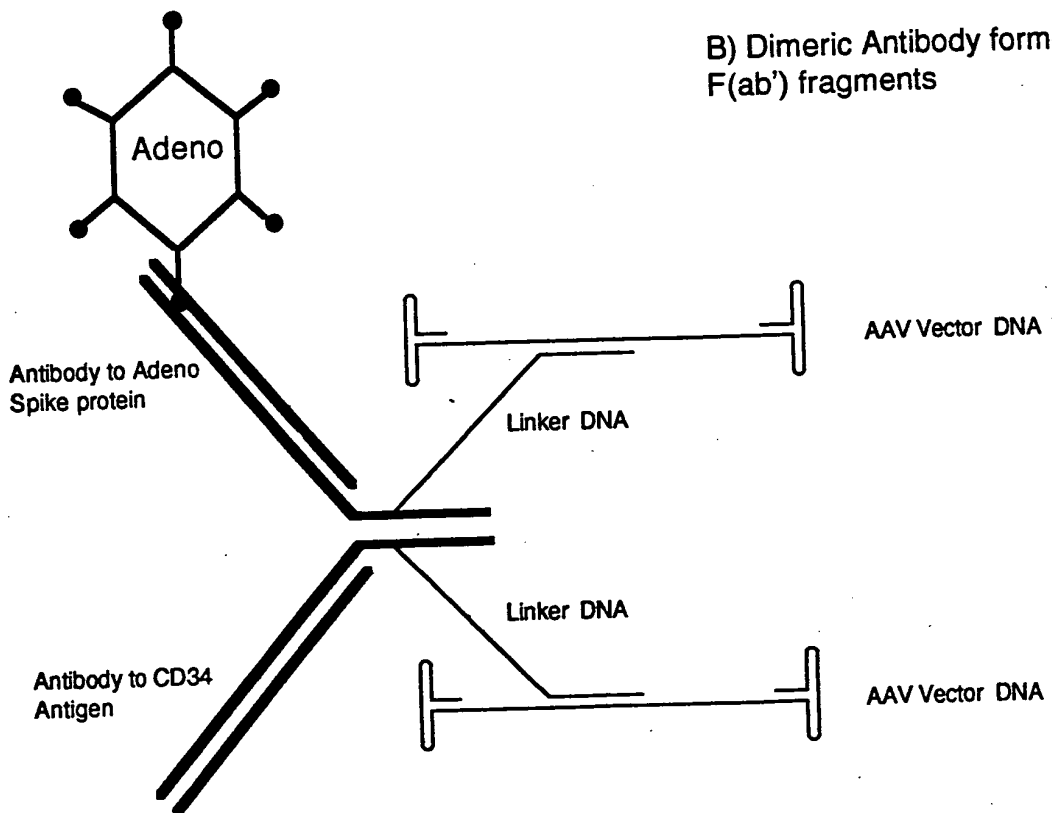
**Figure 15**

Enhanced Delivery of Vector  
DNA to Haematopoietic Stem Cell

A) Dimeric Antibody formed from complete Antibody pieces



B) Dimeric Antibody formed from F(ab') fragments

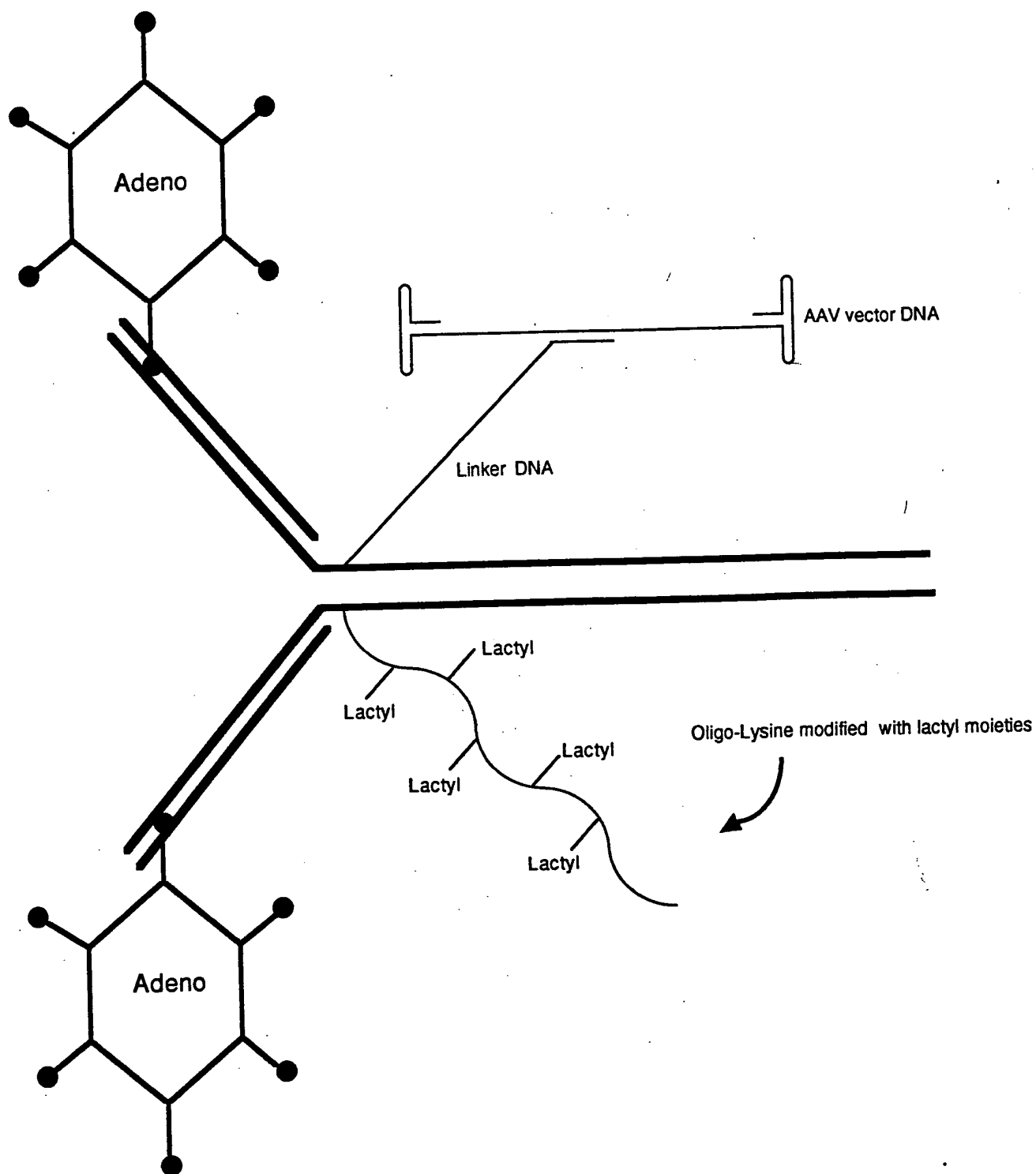


**Figure 16**

Covalent Attachment of vector DNA to Dimeric Antibody

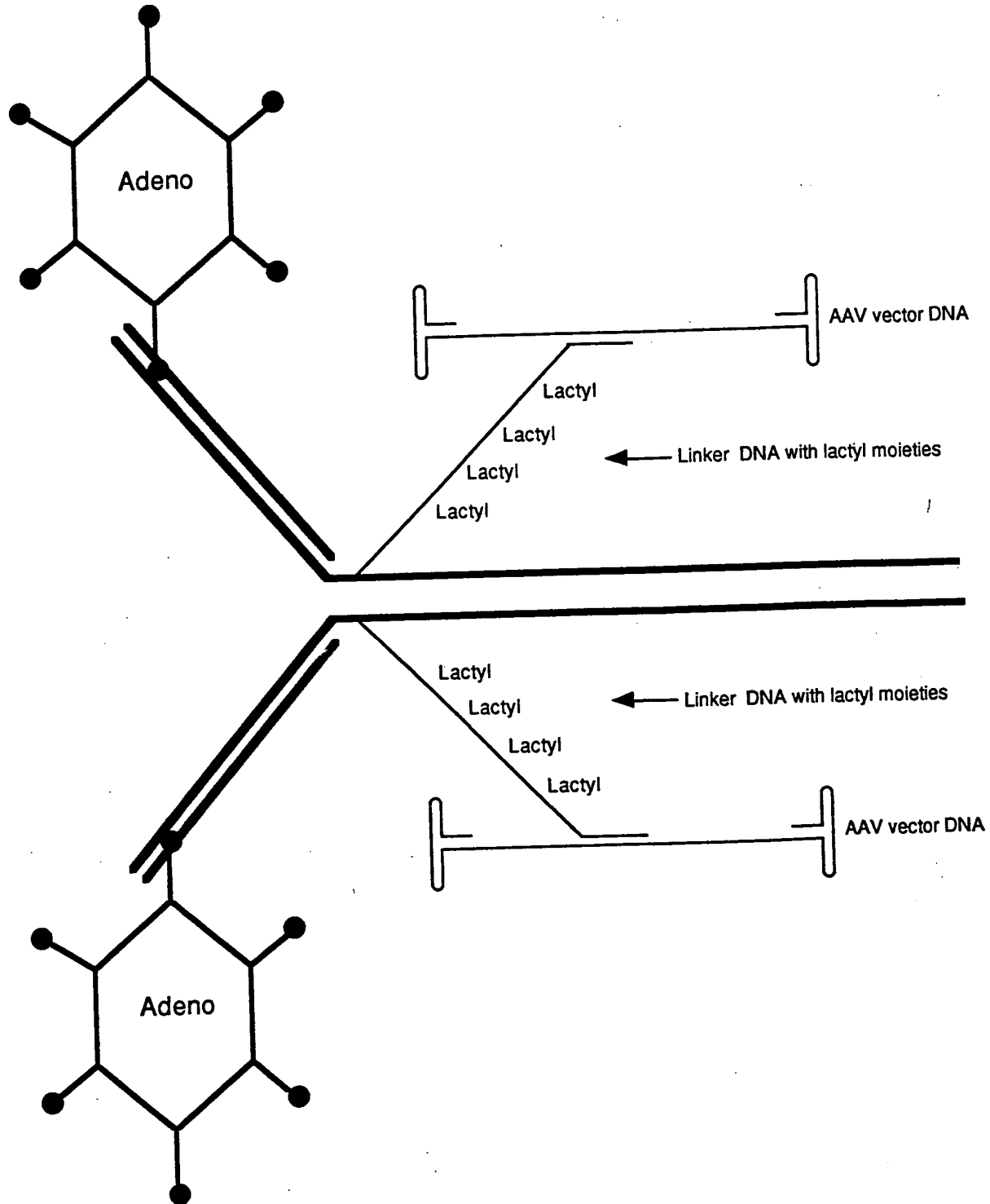
08978632-112597



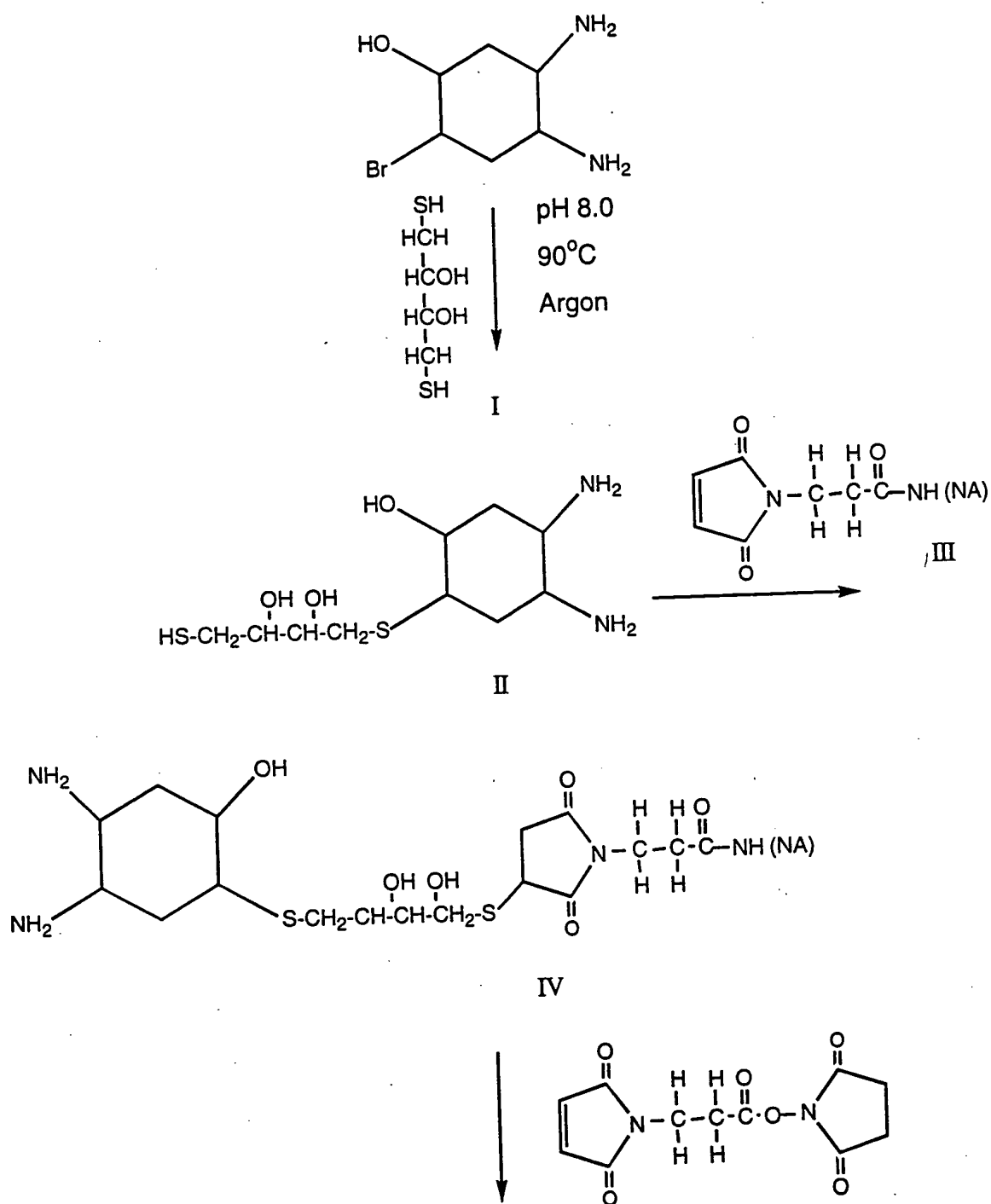


**Figure 17**

Covalent attachment of Modified DNA  
to a Monovalent Antibody



**Figure 18**  
Modified DNA used as a Binder



(continued in Figure 20)

**Figure 19**  
**Synthetic Steps for Creation of Antibodies**  
**With Nucleic Acid Moieties Attached**

(Continued from Figure 19)

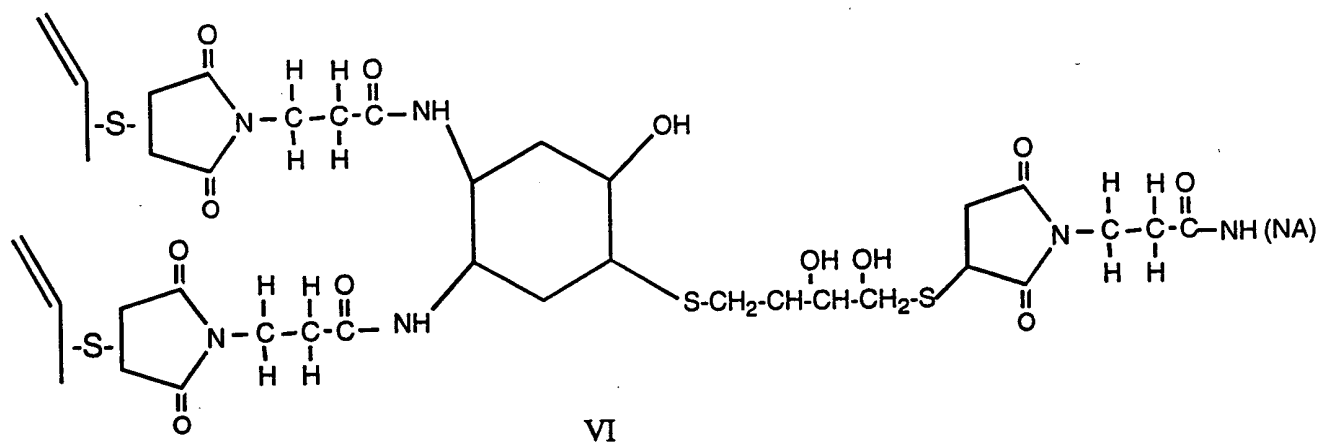
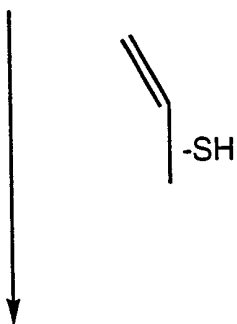
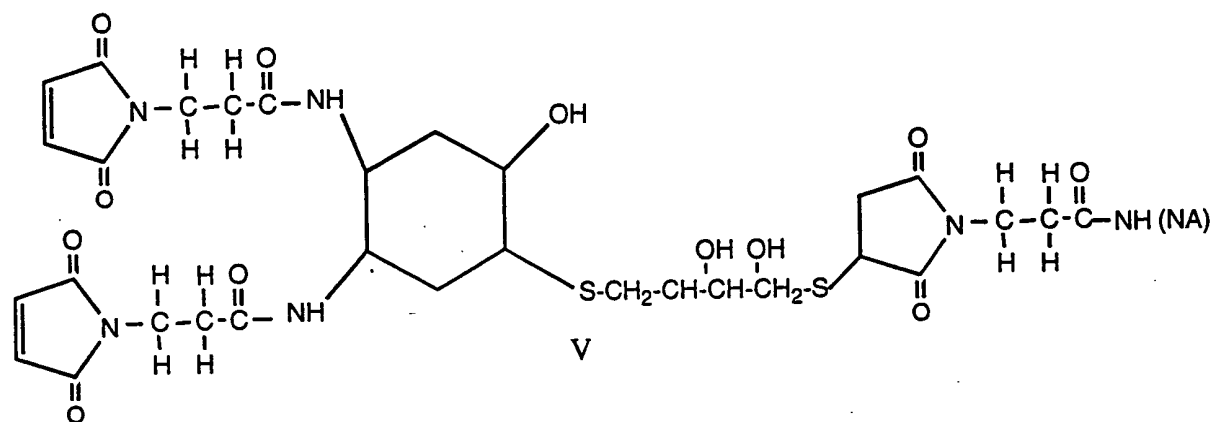
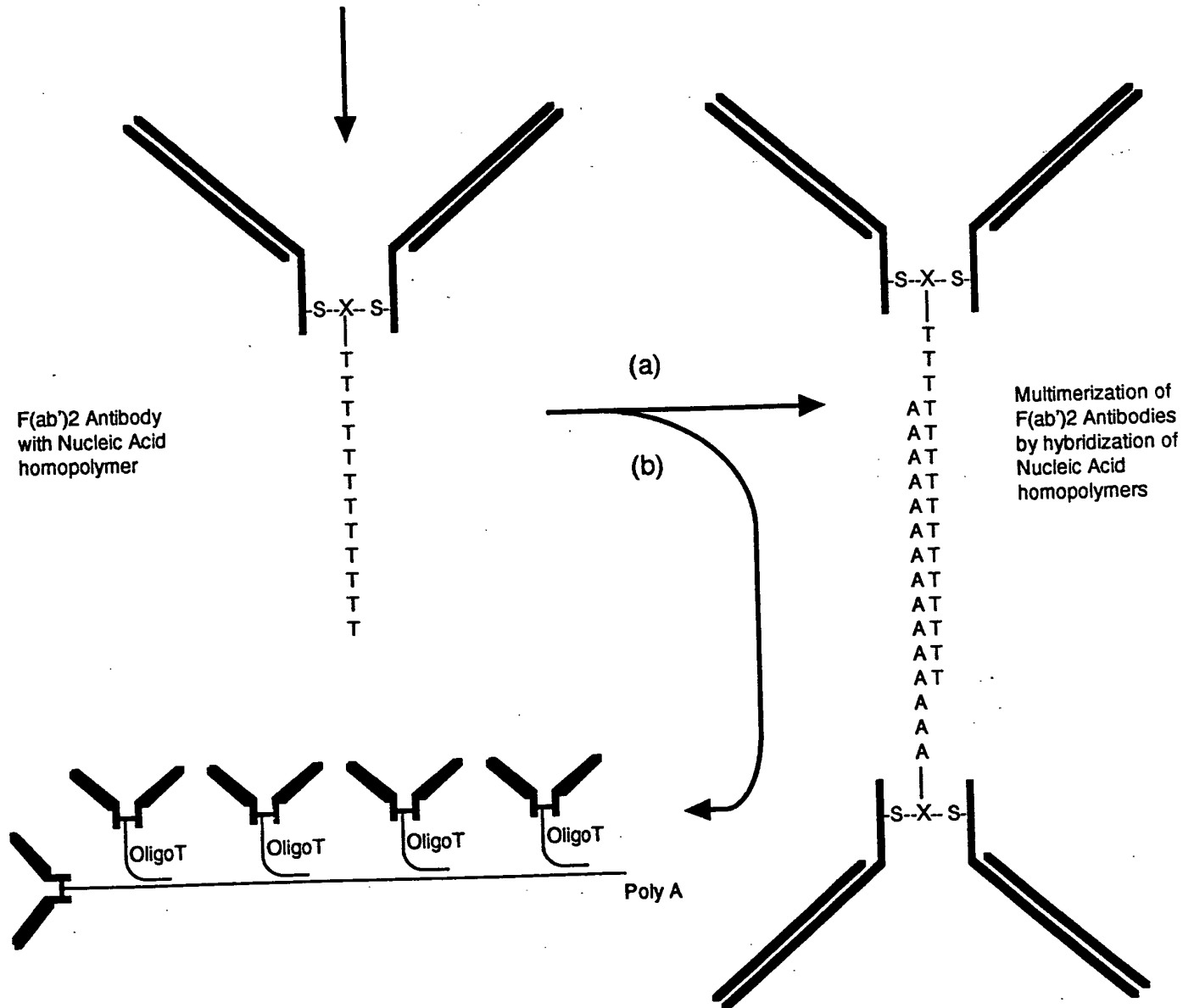


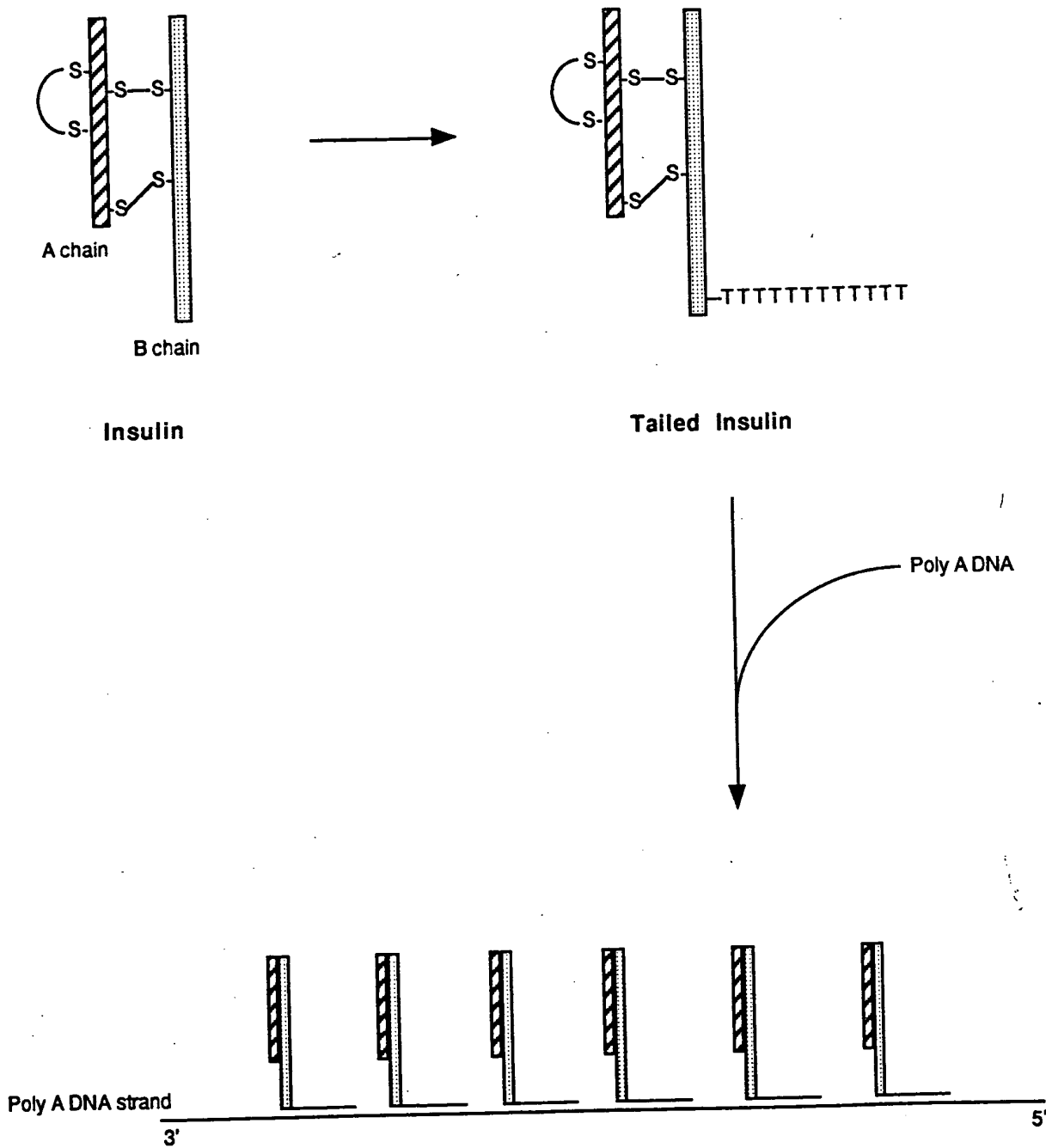
Figure 20  
Continuation of Synthetic Steps

F(ab')<sub>2</sub> Antibody  
to HIV p24  
Antigen

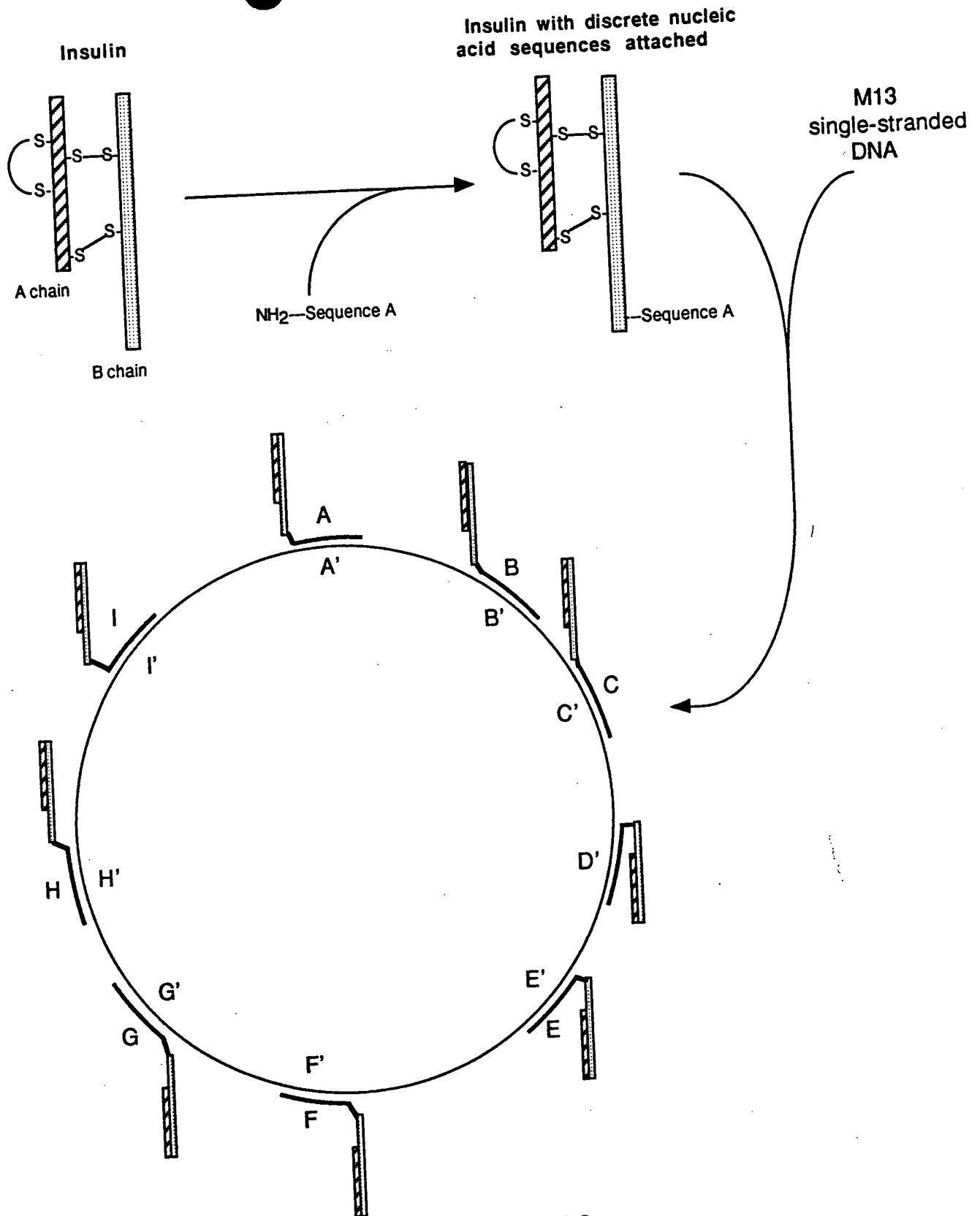


**Figure 21**

Enhanced Binding of Antibodies to Antigens by Multimerization



**Figure 22**  
**High Affinity Multi-Insulin Soluble Complex**



**Figure 23**

Multimerization of Insulin molecules by hybridization to discrete Sequences

08978632-113597

Intron insertion site  
↓

(A)    ---TGCTCTCTAAGGGTCTACTC---  
       ---ACGAGAGATTCCAGATGAG---

T7 RNA Polymerase Sequence

Splice Donor Site  
↓Splice Acceptor site  
↓

(B)    ---CTCTAAGGTAAATAT - - - - - TGTATTTTAGATTCAA---  
       ---GAGATTCATTATA - - - - - ACATAAAATCTAAGTT---

SV40 Intron Sequence

(C)    ---TGCTCTCTAAGGTAAATAT - - - - - TGTATTTTAGGGTCTACTC---  
       ---ACGAGAGATTCCATTATA - - - - - ACATAAAATCCAGATGAG---

Insertion of SV40 Intron into polymerase coding sequence

Splice Donor Site  
↓Splice Acceptor site  
↓

(D)    ---UGCUCUCUAAGGUAUAUAU - - - - - UGUUUUUAGGGUCUACUC---

mRNA transcript containing intron

(E)    ---UGCUCUCUAAGGGUCUACUC---

mRNA transcript after splicing has normal T7 Sequence

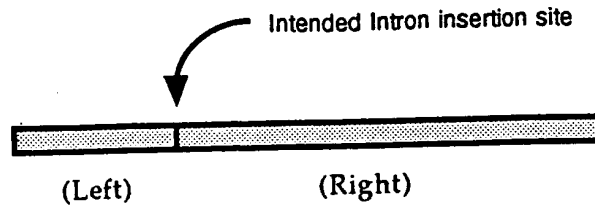
Figure 24

Fusion of Intron into T7 RNA Polymerase Coding Sequence

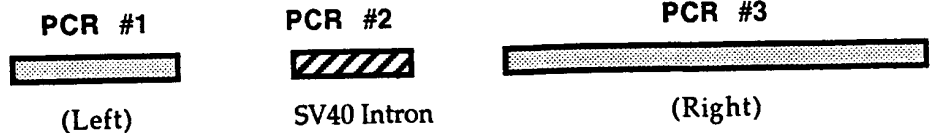
08978332-113597  
26527-23382680



Normal T7 RNA polymerase  
coding sequence

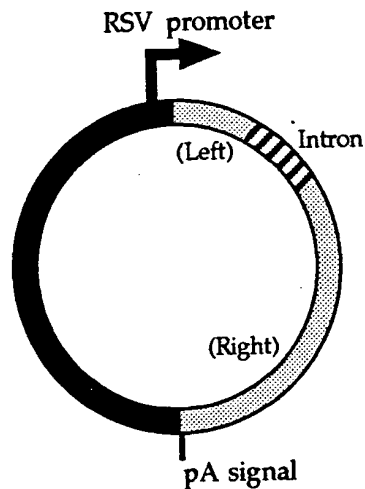


Synthesis of fragments by  
PCR Amplification of T7 or  
SV40 templates



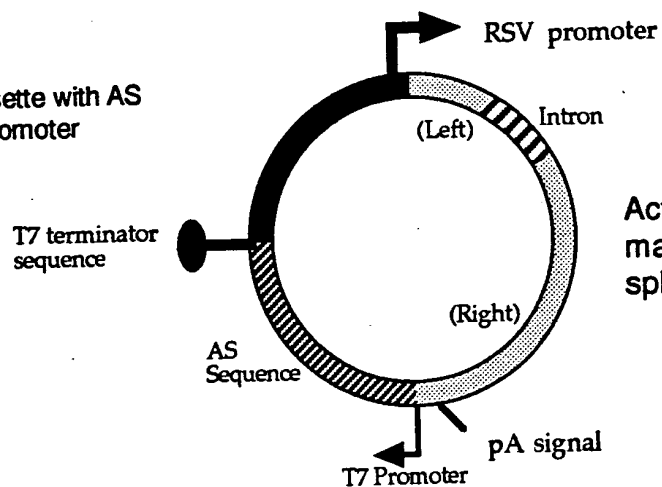
(A)

Fusion of PCR fragments  
together in eucaryotic  
expression vector



(B)

Introduction of cassette with AS  
directed from T7 promoter



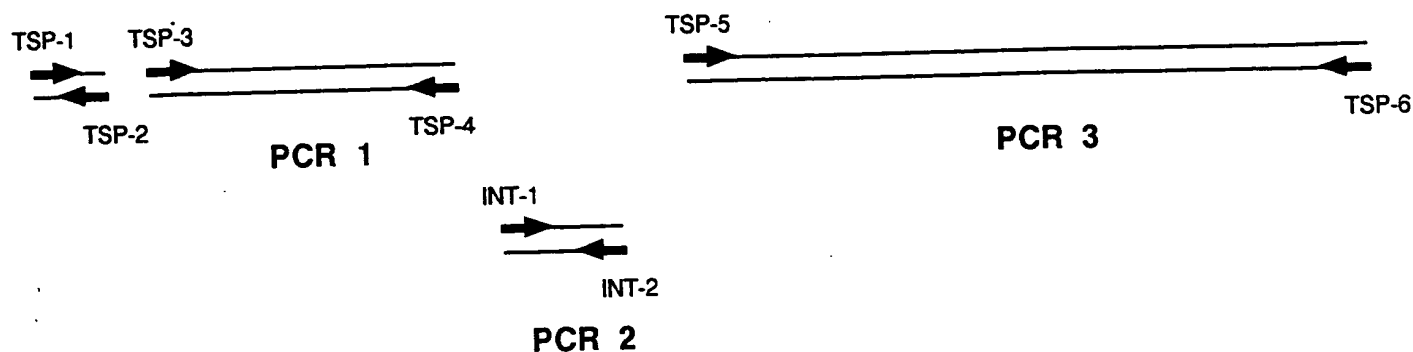
(C)

Active T7 RNA polymerase is only  
made in eucaryotic cells after  
splicing out of SV40 Intron

**Figure 25**

**Construction of T7 Expression Vector**

## A) Synthesis of pieces



## B) Oligomers used for synthesis

TSP-1	GGA ATT CGT CTC GAG CTC TGA TCA CCA CCA TGG ACA CGA TTA ACA TCG C
TSP-2	GAC TAG TTG GTC TCG TCT CTT TTT TGG AGG AGT GTC GTT CTT AGC GAT GTT AAT C
TSP-3	GGA ATT CGT CTC GGA GAA AGG TAA AAT TCT CTG ACA TCG AAC TGG C
TSP-4	GAC TAG TGG TCT CCC CTT AGA GAG CAT GTC AGC
TSP-5	GGA ATT CGG TCT CGG GTC TAC TCG GTG GCG AGG
TSP-6	GAC TAG TCG TTA CGC GAA CGC AAA GTC
INT-1	GGA ATT CGT CTC TAA GGT AAA TAT AAA ATT TTT AAG
INT-2	GAC TAG TCG TCT CTG ACC CTA AAA TAC ACA AAC AAT TAG A

Figure 26

Synthesis of Pieces for Construction of  
T7 RNA Polymerase with Intron

# Formation of Nuclear Localisation Signal by Fusion of TSP1/TSP2 Product to Clone with PCR #1 product

## Annealing of TSP1 with TSP2

TSP1

5' GG AAT TCG TCT CGA GCT CTG ATC ACC ACC ATG GAC ACG ATT AAC ATC GC 3' TSP2  
3' C TAA TTG TAG CGA TTC TTG CTG TGA GGA GGT TTT TTC TCT GCT CTG GTT GAT CAG 5'

## Extension of TSP1/TSP2 by polymerase

5' GG AAT TCG TCT CGA GCT CGA GAC GTA TGG TGG TAC CTG TGC TAA TTG TAG CGA TTC TTG CTG TGA GGA GGT TTT TTC TCT GAT CTA GTC 3'  
3' CC TTA AGC AGA GCT CGA GAC GTA TGG TGG TAC CTG TGC TAA TTG TAG CGA TTC TTG CTG TGA GGA GGT TTT TTC TCT GAT CAG 5'

Bsa I

## Digestion of TSP1/TSP2 product with Bsa I

5' GG AAT TCG TCT CGA GCT CTG ATC ACC ACC ATG GAC ACG ATT AAC ATC GCT AAG AAC GAC ACT CCT CCA AAA AA  
3' CC TTA AGC AGA GCT CGA GAC GTA TGG TGG TAC CTG TGC TAA TTG TAG CGA TTC TTG CTG TGA GGA GGT TTT TTC TCT

## Digestion of PCR #1 clone (pL-1) with BsmB I

Bsm B1  
5' GGA ATT CGT CTC G GAGA AAG GTA AAA TTC TCT GAC ATC GAA CTG GC-----  
CCT TAA GCA GAG CCTCT TTC CAT TTT AAG AGA CTG TAG CTT GAC CG-----

## Ligation of Bsa I digested TSP1/TSP2 product to BsmB I digested PCR#1 clone

5' GG AAT TCG TCT CGA GCT CTG ATC ACC ACC ATG GAC ACG ATT AAC ATC GCT AAG AAC GAC ACT CCT CCA AAA AAG AGA AAG GTA AAA TTC  
3' CC TTA AGC AGA GCT CGA GAC GTA TGG TGG TAC CTG TGC TAA TTG TAG CGA TTC TTG CTG TGA GGA GGT TTT TTC TCT TTC CAT TTT AAG  
TCT GAC ATC GAA CTG GC-----  
AGA CTG TAG CTT GAC CG-----

Figure 27

# Comparison of the 5' ends of the Nucleotide Sequences of Wild Type and Modified T7 RNA Polymerase

## Wild Type T7 nucleic and amino acid sequence

ATG	GAC	ACG	ATT	AAC	ATC	GCT	ATG	AAC	GAC	TTC	TCT	GAC	ATC	GAA	CTG	GC	-----
TAC	CTG	TGC	TAA	TTG	TAG	CGA	TTT	CTG	AAG	AGA	CTG	TAG	CTT	GAC	CG	-----	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		

## Modified T7 nucleic and amino acid sequence with Nuclear Localisation Signal (NLS) insertion

ATG	GAC	ACG	ATT	AAC	ATC	GCT	ATG	AAC	GAC	ACT	CCT	CCA	AAA	AAG	AGA	AAG	GTA	AAA	TTC	TCT	GAC	ATC	GAA	CTG	GC	-----	
TAC	CTG	TGC	TAA	TTG	TAG	CGA	TTT	CTG	TTC	TTC	TTC	TTC	TTC	TTC	TTC	TTC	TTC	TTC	TTC	TTC	TTC	TTC	TTC	TTC	TTC	TTC	-----
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	-----	

Figure 28

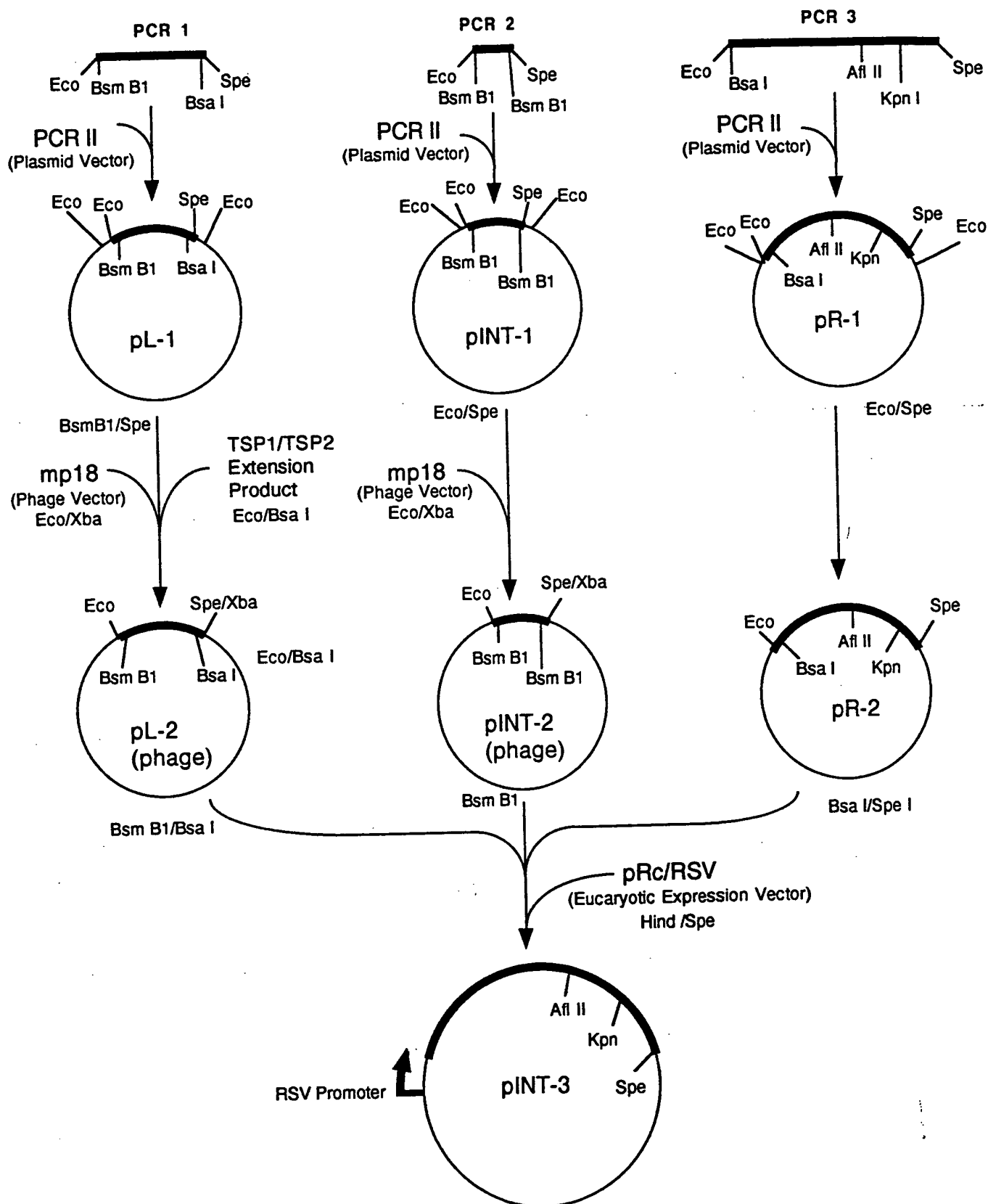


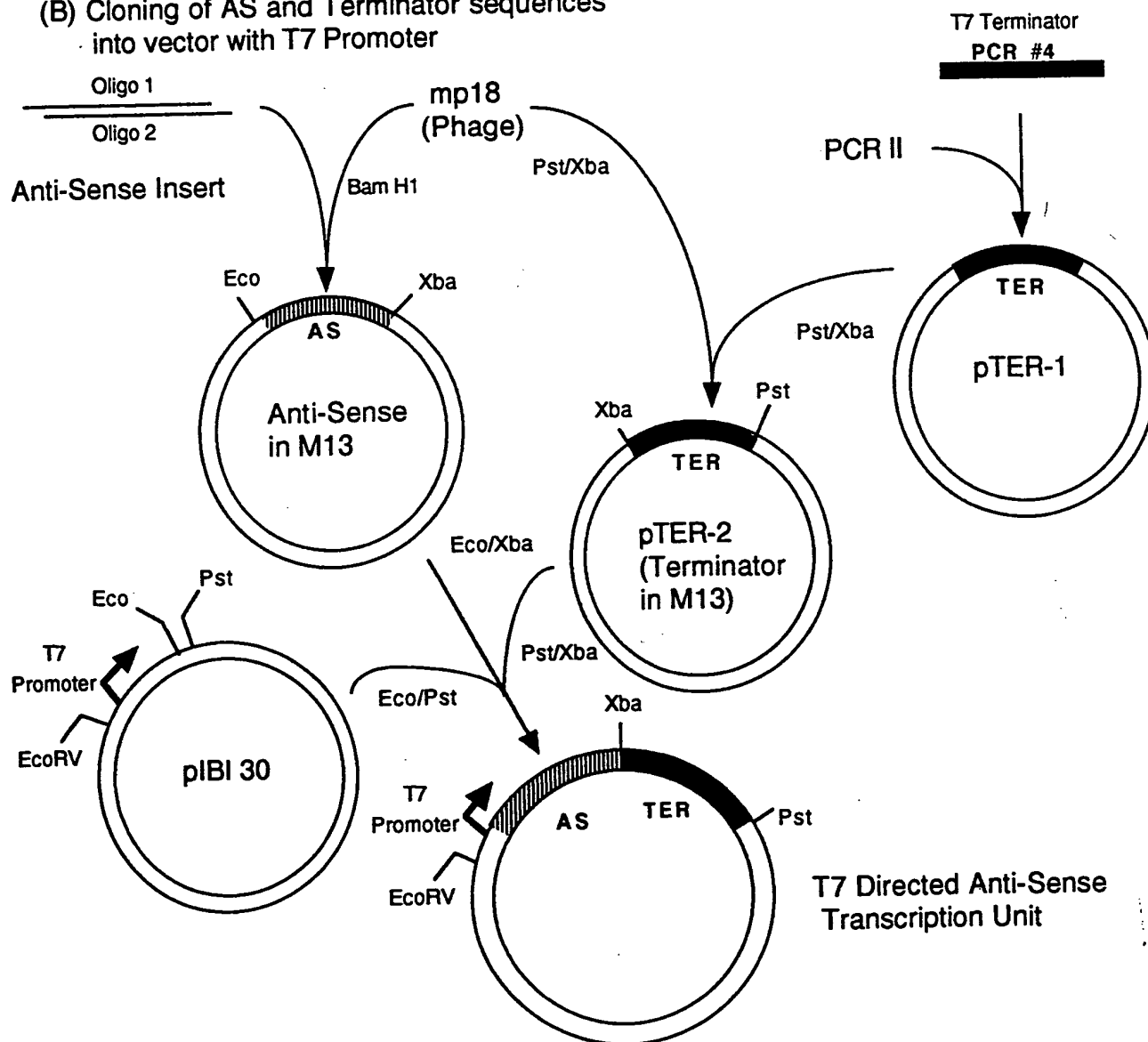
Figure 29

Fusion of PCR Pieces to Construct  
T7 RNA Polymerase with an Intron

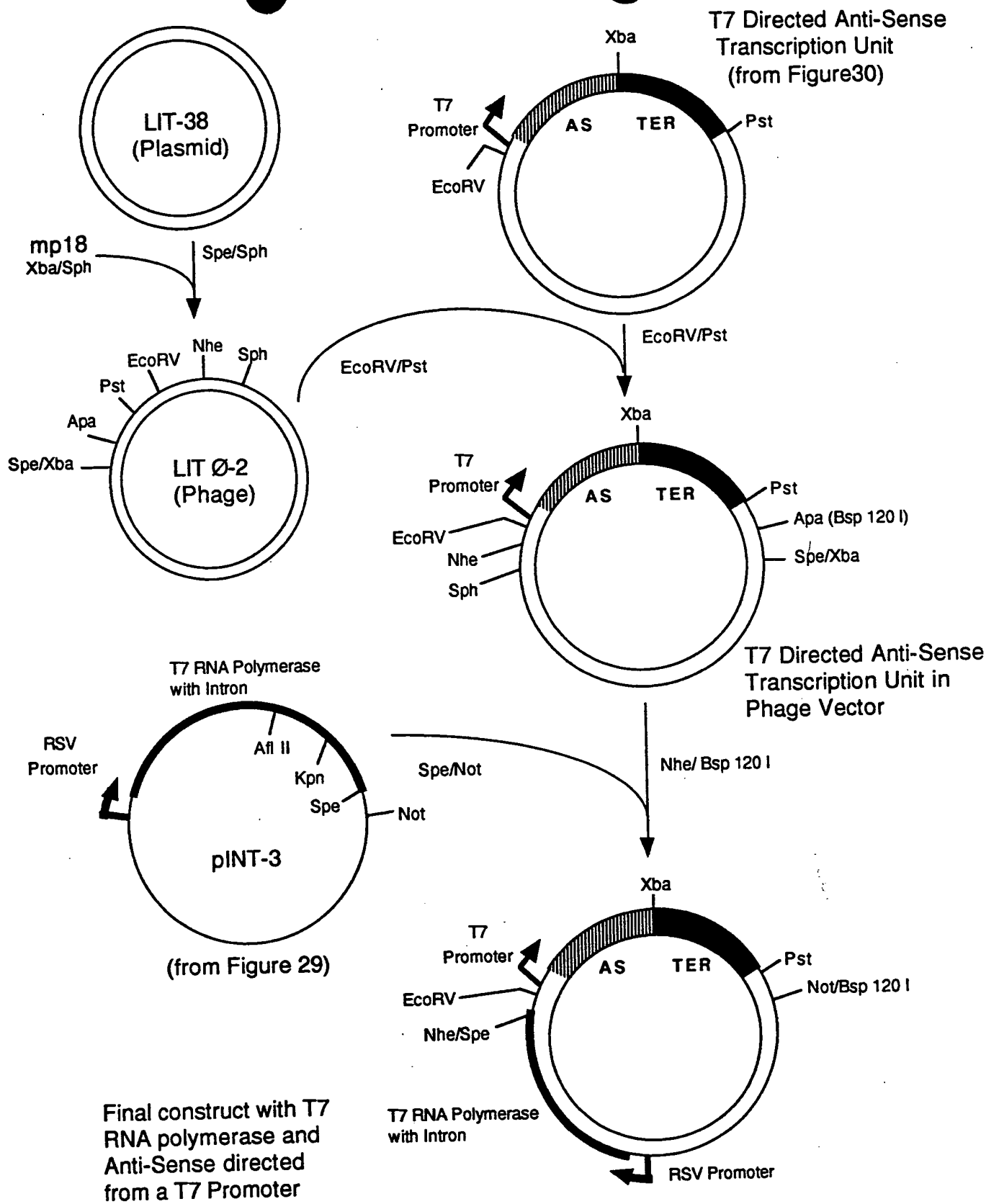
## (A) Oligomers

HTA-1	GAT CAT TAG ACC AGA TCT GAG CCT GGG AGC TCT CTG GCT AAC TAG GGA ACC CAC TGCTTA AGC CTC AAG
HTA-2	GAT CCT TGA GGC TTA AGC AGT GGG TTC CCT AGT TAG CCA GAG AGC TCC CAG GCT CAG ATC TGG TCT AAT
HTB-1	GAT CAC CTT AGG CTC TCC TAT GGC AGG AAG AAG CGG AGA CAG CGA CGA AGA CCT CCT CAA G
HTB-2	GAT CCT TGA GGA GGT CTT CGT CGC TGT CTC CGC TTC TTC CTG CCA TAG GAG AGC CTA AGG T
HTC-1	GAT CAT AGT GAA TAG AGT TAG GCA GGG ATA CTC ACC ATT ATC GTT TCA GAC CCA CCT CCC AG
HTC-2	GAT CCT GGG AGG TGG GTC TGA AAC GAT AAT GGT GAG TAT CCC TGC CTA ACT CTA TTC ACT AT
TER-1	AAT CTA GAG CTA ACA AAG CCC GAA AGG AAG
TER-2	TTC TGC AGA TAT AGT TCC TCC TTT CAG C

## (B) Cloning of AS and Terminator sequences into vector with T7 Promoter

**Figure 30**

Insertion of Anti-Sense Sequences into  
T7 Directed Transcription Units



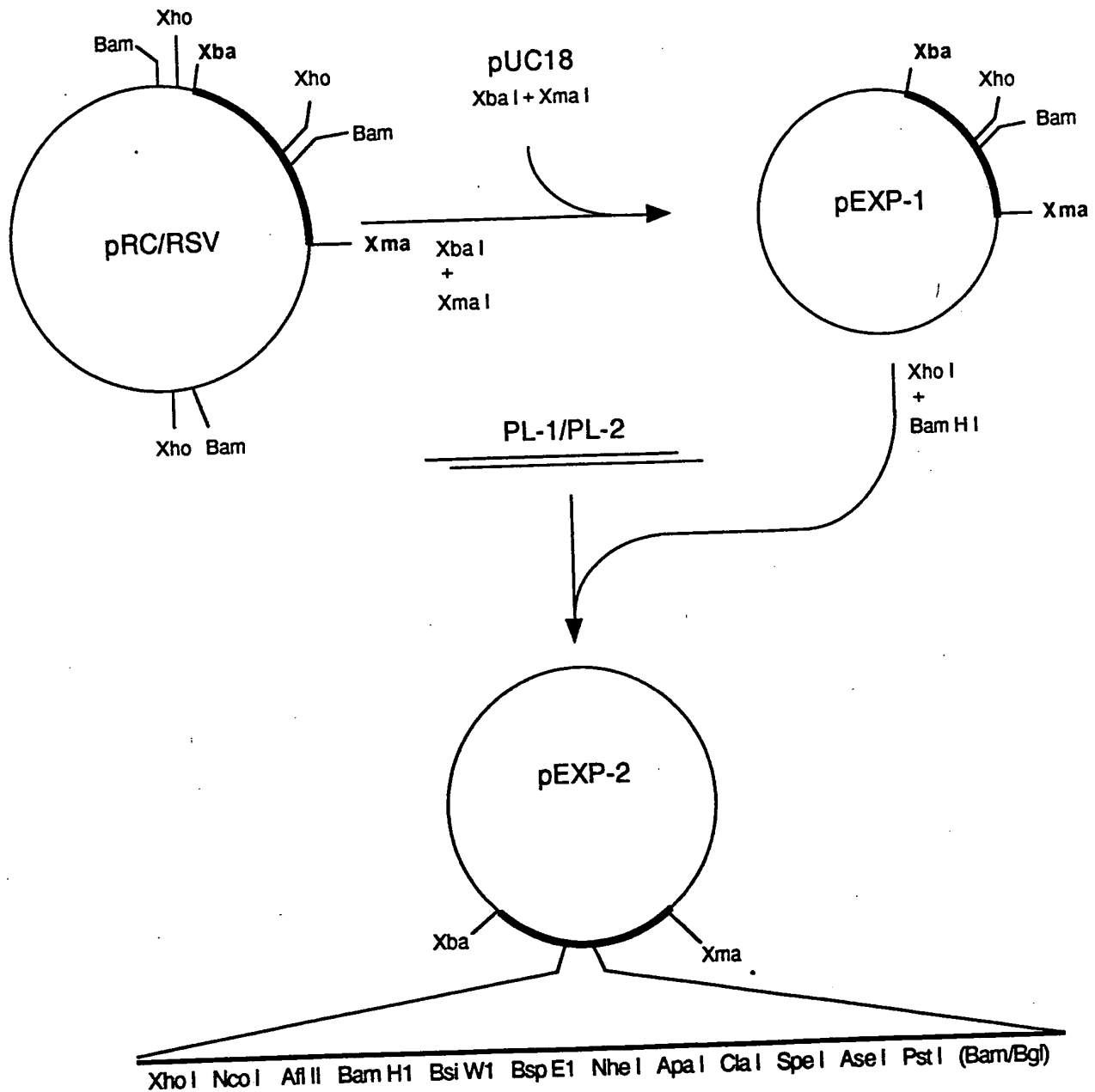
**Figure 31**

Construct with T7 RNA polymerase and Anti-Sense directed from a T7 Promoter

# A) Oligomers for introduction of T7 signals and polylinker

PL-1 TCG AGC CAT GGC TTA AGG ATC CGT ACG TCC GGA GCT AGC GGG CCC ATC GAT ACT  
AGT TAA ATG CAG ATC T

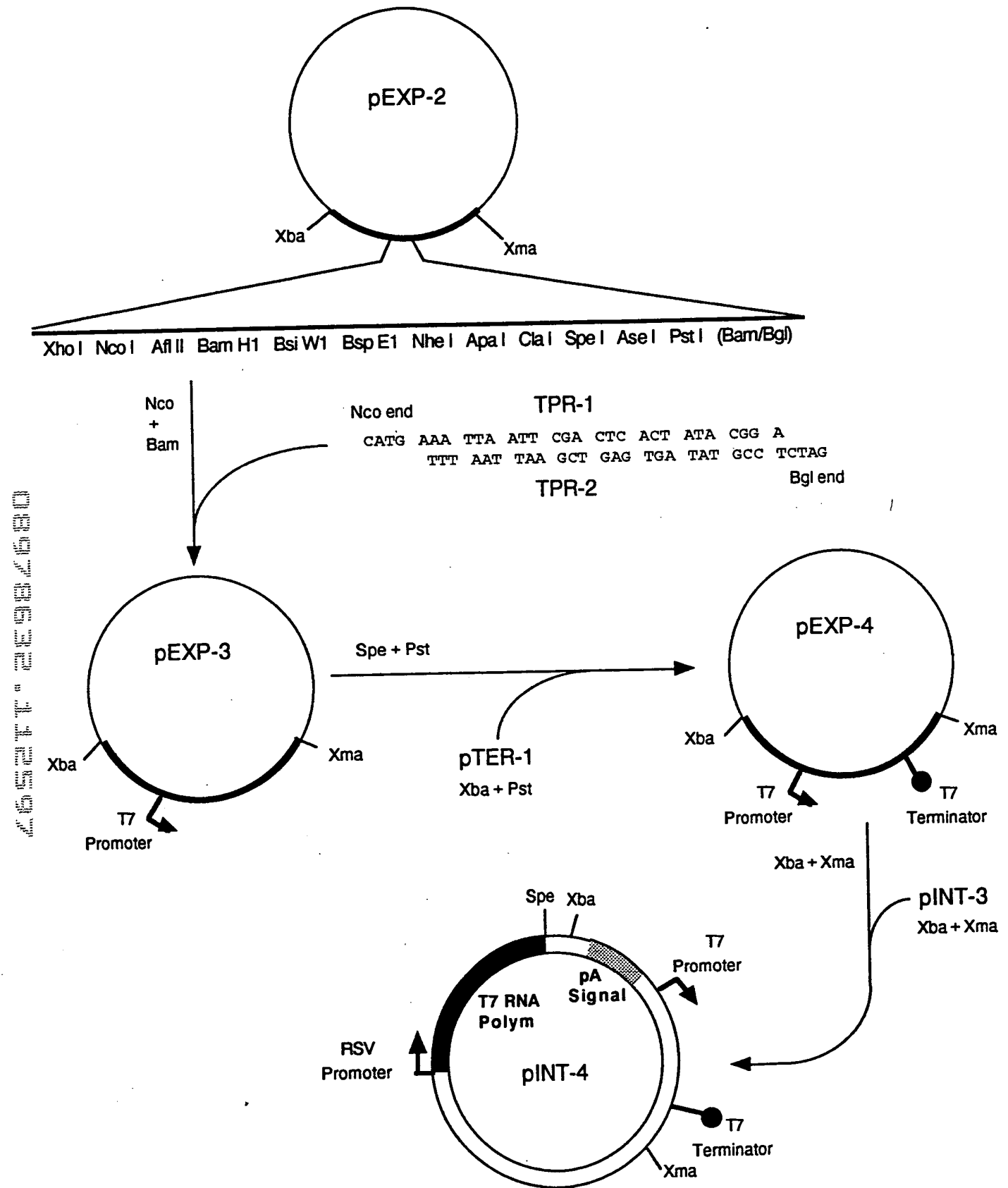
PL-2 CTA GAG ATC TGC ATT TAA CTA GTA TCG ATG GGC CCG CTA GCT CCG GAC GTA CGG  
ATC CTT AAG CCA TGG C



**Figure 32**

Introduction of Poly-Linker for Creation of Protein Expression Vector



**Figure 33**

Final steps for construction of Expression Vector

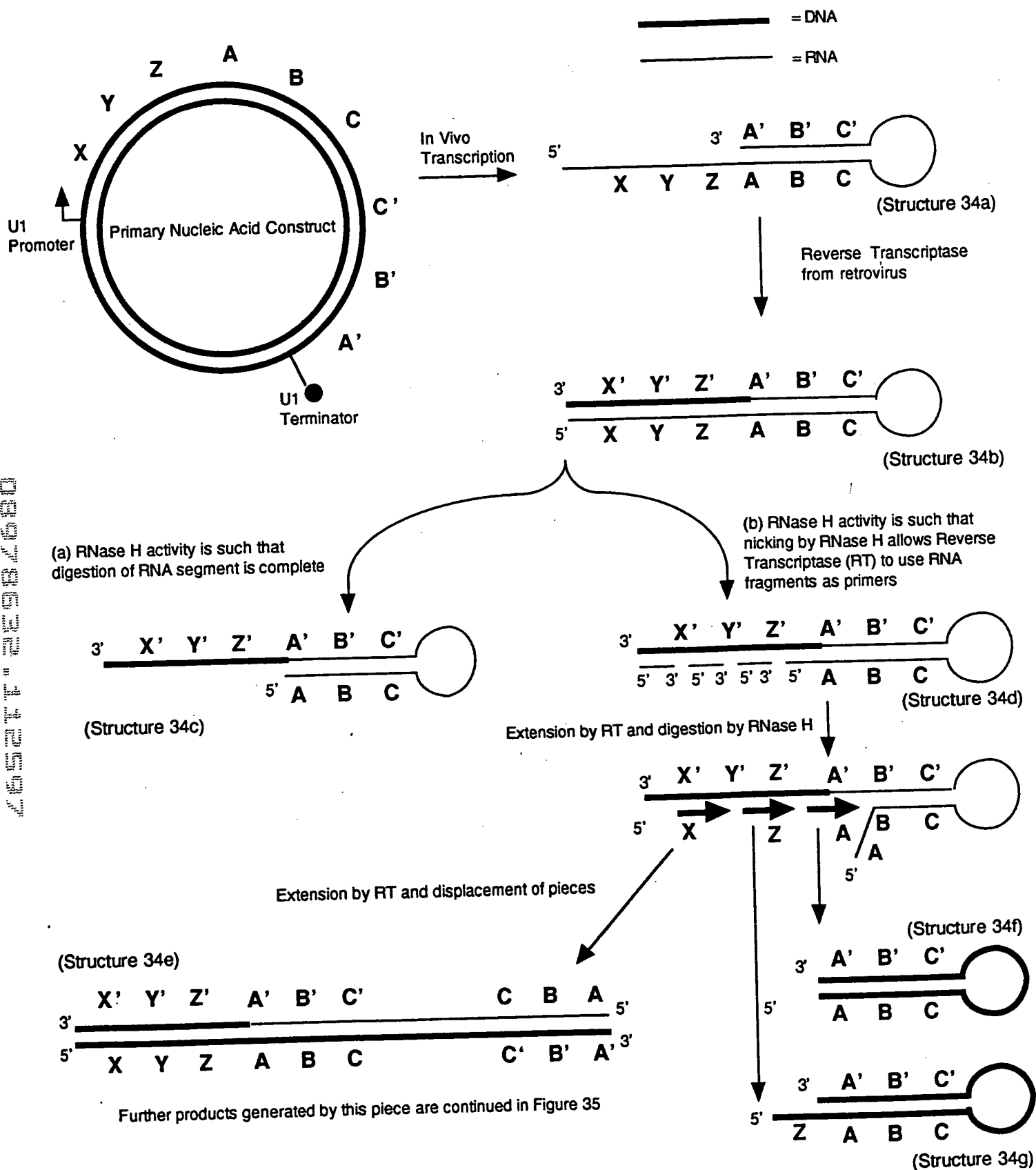
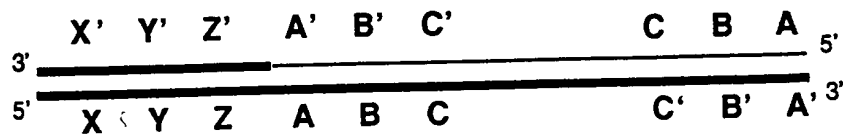


Figure 34

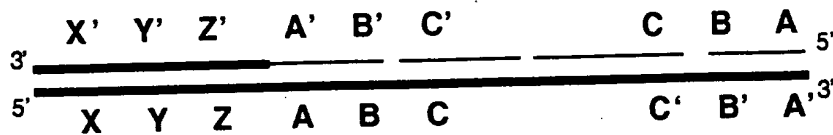
Construct that produces single-stranded Anti-Sense DNA

Continued from Figure 34

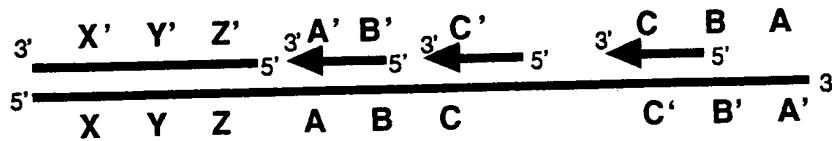
(Structure 34e)



Nicking by RNase H



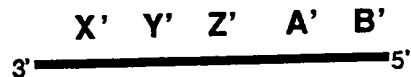
Extension by RT and digestion by RNase H



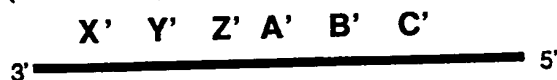
(Structure 35h)



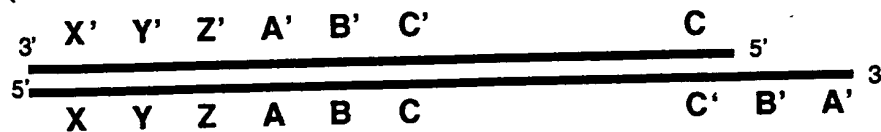
(Structure 35i)



(Structure 35j)



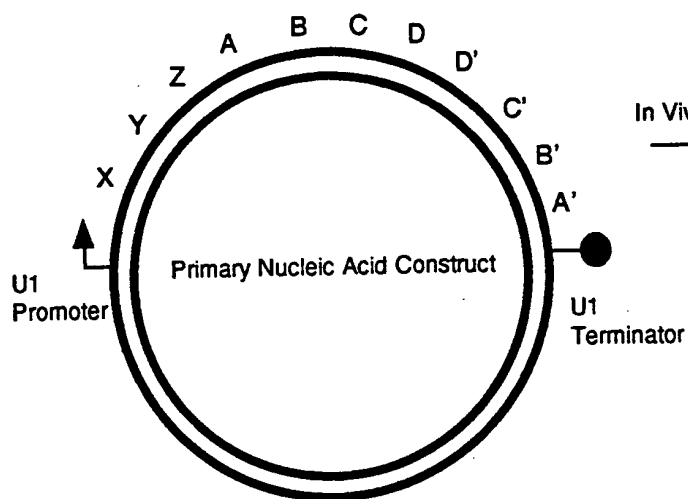
(Structure 35k)



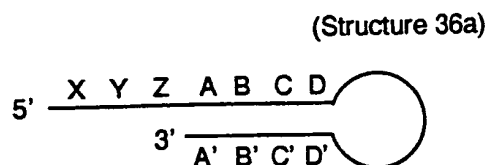
Extension by RT and displacement generates Single-Stranded DNA and a mostly Double-stranded DNA molecule

**Figure 35**  
Continuation of Process from Figure 34

2025-11-26 14:26:26



In Vivo Transcription

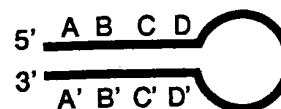


In a series of events similar to that shown for Example G-1, the net products of Rnase H and RT activities on the transcript above create Double-stranded DNA products similar to these below:

= DNA  
 = RNA

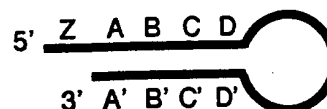
In this example, A B C is a promoter sequence, directing transcription off of these Double-stranded DNA products to create RNA transcripts with varying amounts of double-stranded character. Furthermore, the single-stranded loop segment (D to D') of the transcript codes for anti-sense sequences

(Structure 36b)



+

(Structure 36c)



+

(Structure 36d)

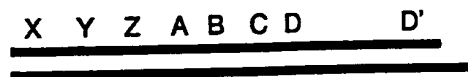
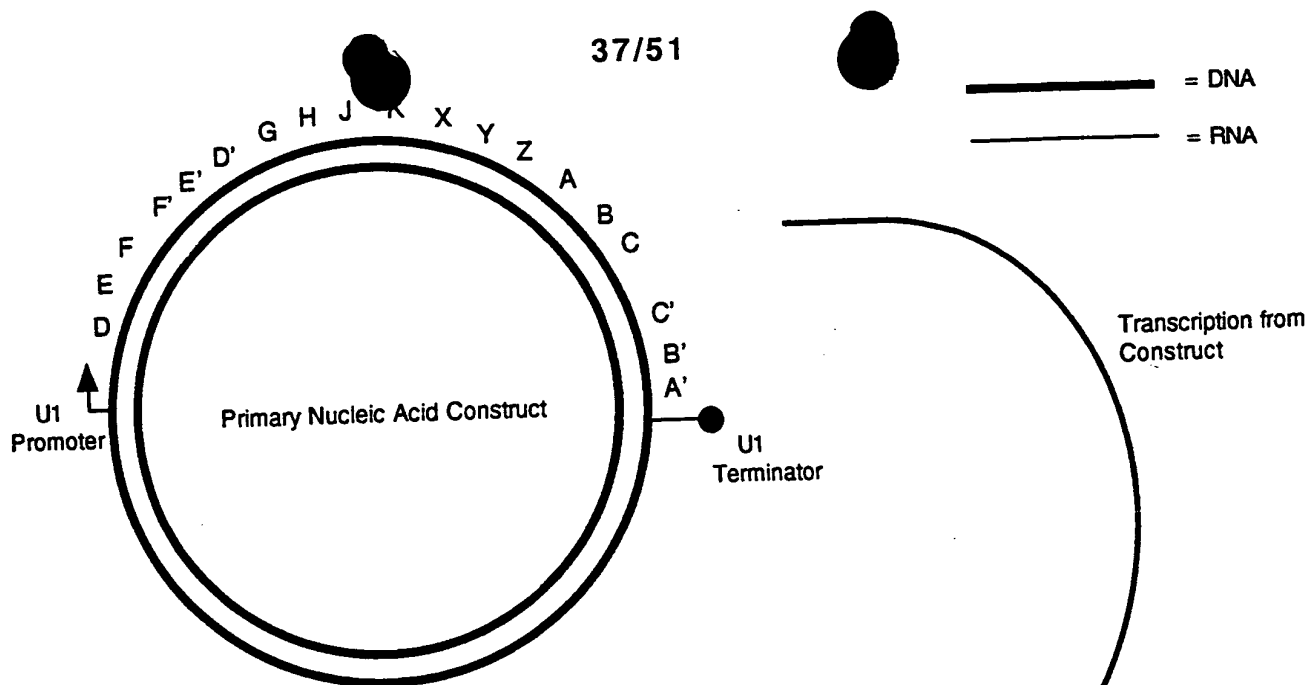


Figure 36

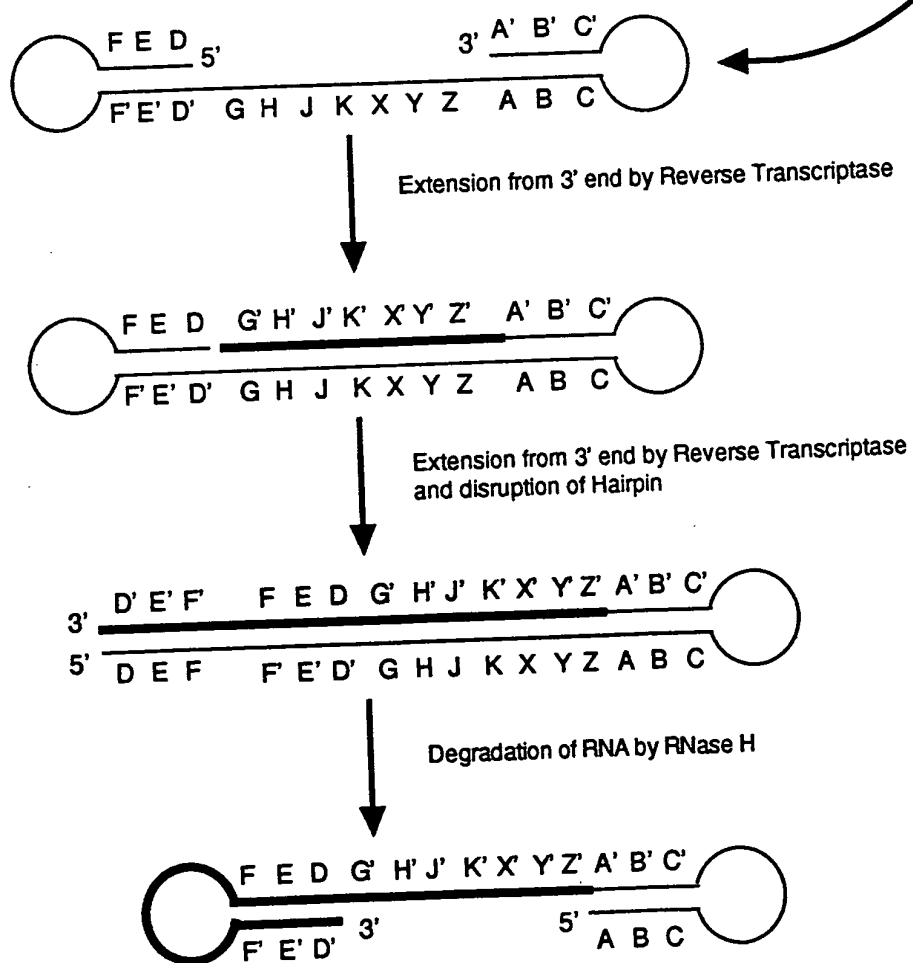
Construct that produces RNA that is Reverse Transcribed to create Secondary DNA Constructs capable of directing transcription

00978632-13597  
265217 265260

— = DNA  
— = RNA



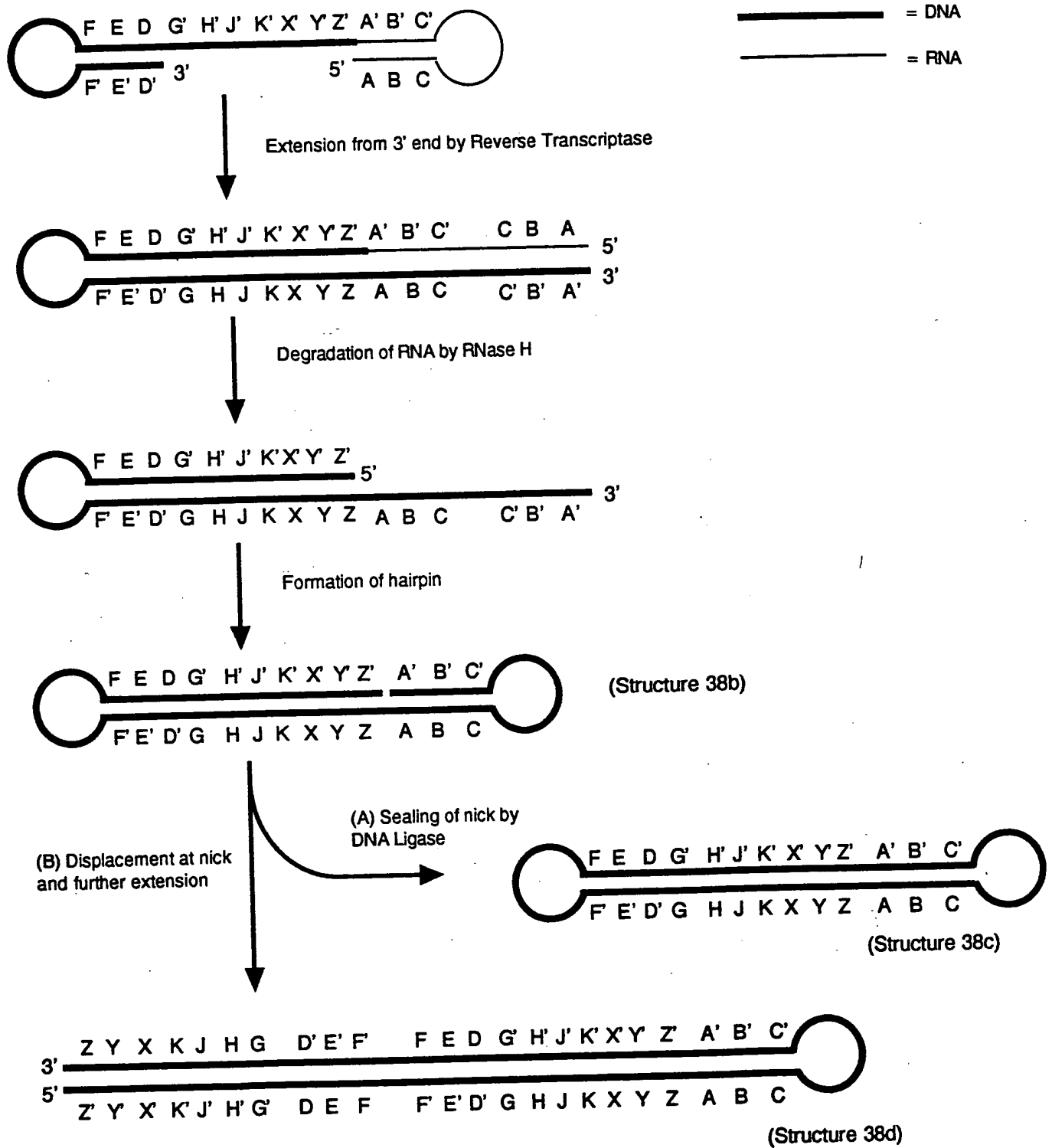
(Structure 37a)



(Continued in Figure 38)

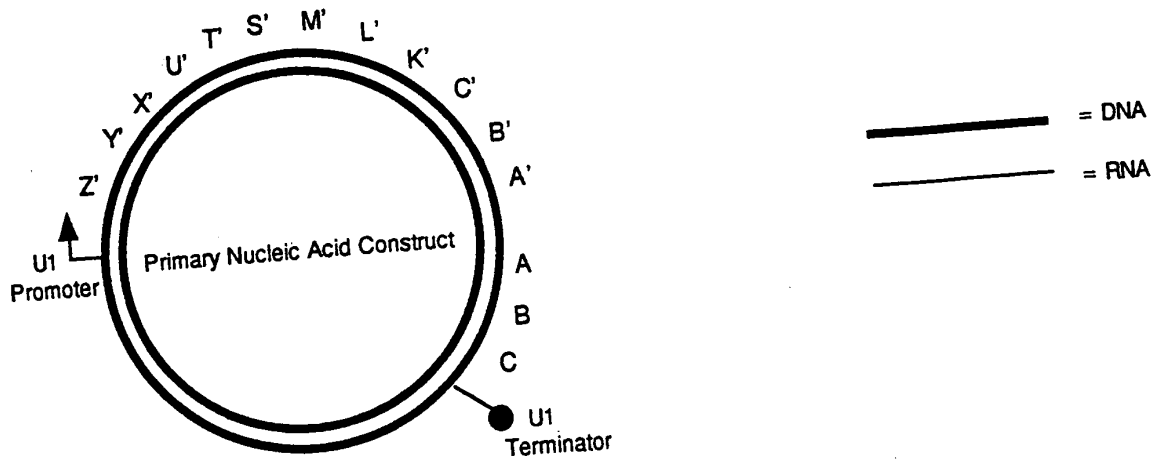
**Figure 37**

Construct which Propagates a Double Hairpin Production Center

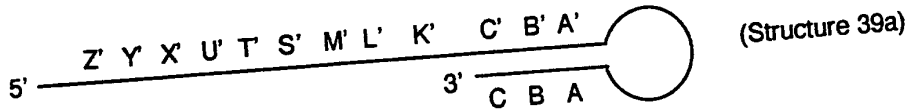


In this Example, the sequence  $F' E' D'$  is a promoter, the sequence  $G H J K$  is an Anti-Sense sequence and  $X Y Z$  is a Poly A signal

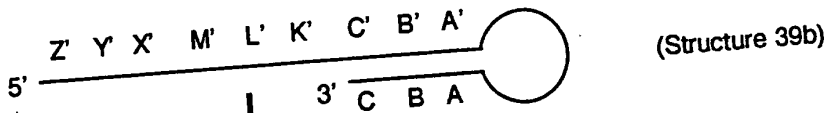
**Figure 38**  
 Continuation of process from Figure 37



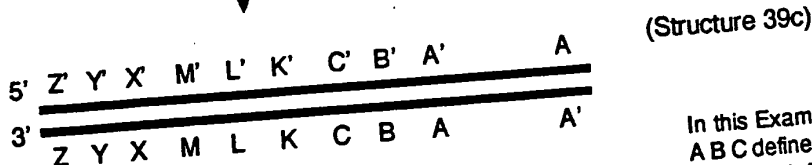
In vivo transcription



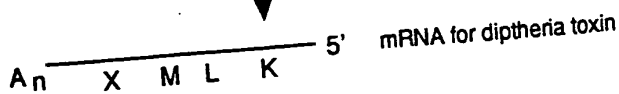
Splicing out of Intron on Anti-coding strand. In this example the sequence X' U' T' S' M' reflects splice donor, intron and splice acceptor sequences.



A series of RNase H and Reverse Transcriptase steps as shown in Figures 34 and 35



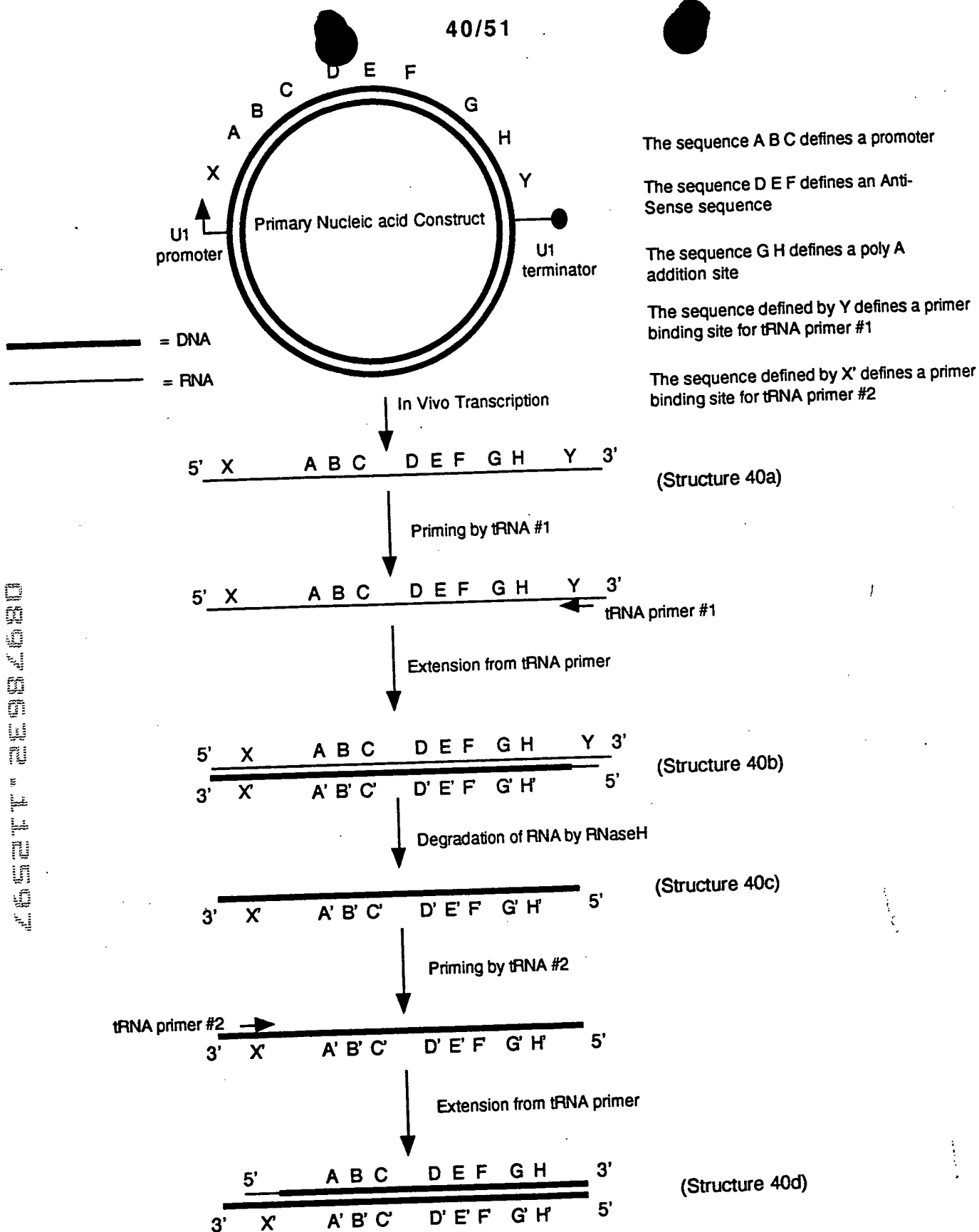
In vivo transcription



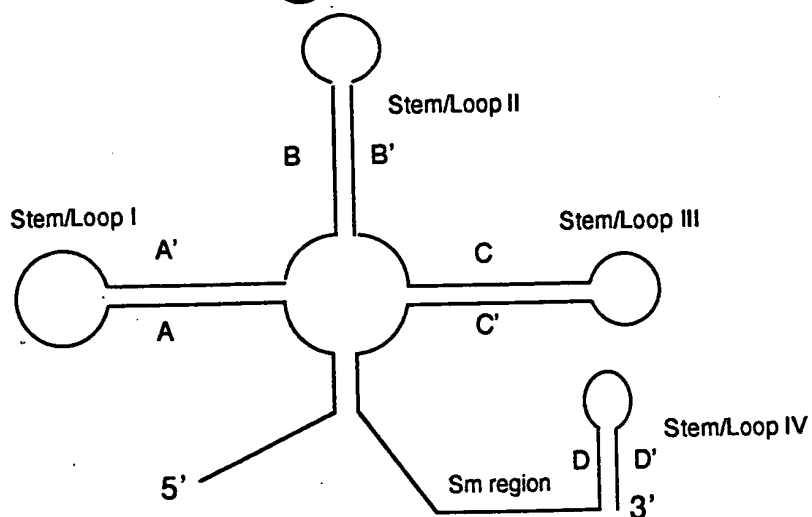
In this Example:  
 A B C defines the sequence of an HIV LTR,  
 K L M X defines the sequence of diptheria toxin  
 YZ defines the sequence for a poly A signal.

Figure 39

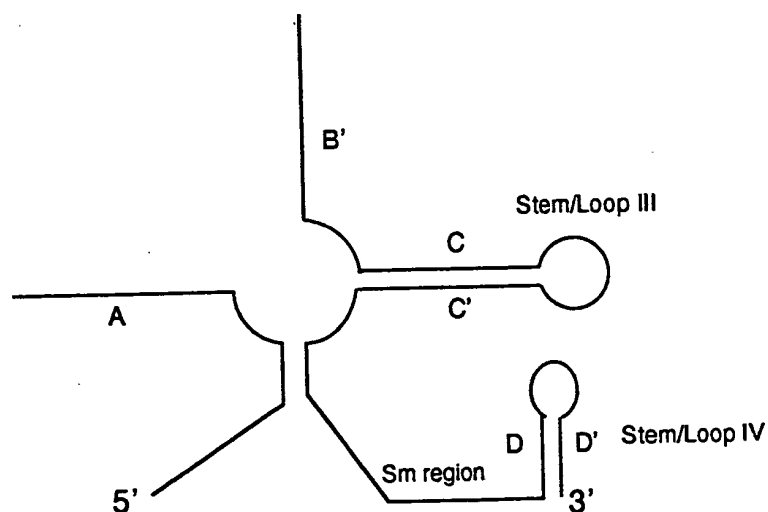
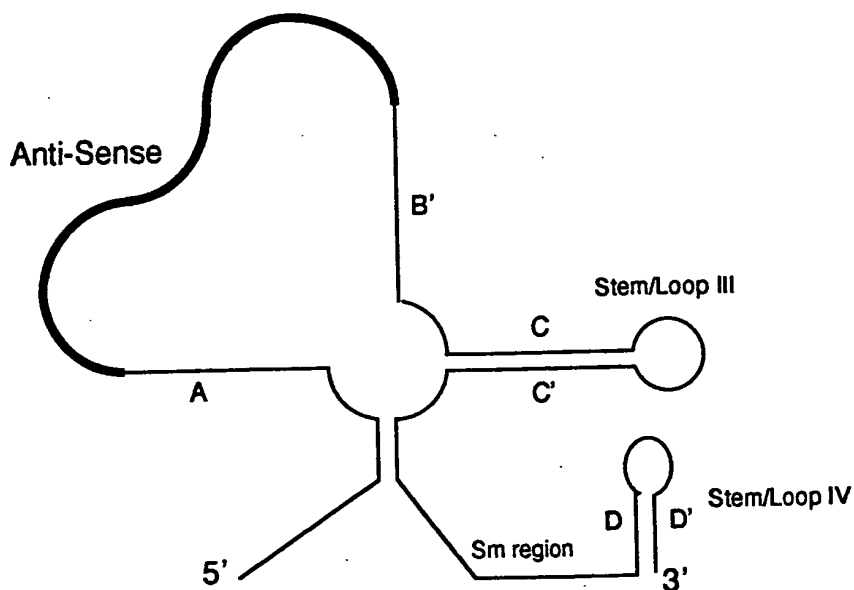
Construct which propagates a Production  
 Center capable of Inducible Suicide







Normal U1

U1 with  
Bcl I/Bsp EI  
piece removedU1 with Anti-Sense  
sequence inserted**Figure 41**

Excision of Sequences from U1 Transcript Region  
and Replacement with Novel Sequences

0897833.13597  
265277 265276

## (A) Anti-sense oligomers

HVA-1 GAT CCG GAT TGA GGC TTA AGC AGT GGG TTC CCT AGT TAG CCA GAG AGC TCC CAG GCT CAG ATC TGG TCT AAT  
HVA-2 CCG GAT TAG ACC AGA TCT GAG CCT GGG AGC TCT CTG GCT AAC TAG GGA ACC CAC TGC TTA AGC CTC AAT CCG

HVB-1 GAT CCG GAC CTT GAG GAG GTC TTC GTC GCT GTC TCC GCT TCT TCC TGC CAT AGG AGA GCC TAA GGT  
HVB-2 CCG GAC CTT AGG CTC TCC TAT GGC AGG AAG AAG CGG AGA CAG CGA CGA AGA CCT CCT CAA GGT CCG

HVC-1 GAT CCG GAT GGG AGG TGG GTC TGA AAC GAT AAT GGT GAG TAT CCC TGC CTA ACT CTA TTC ACT AT  
HVC-2 CCG GAT AGT GAA TAG AGT TAG GCA GGG ATA CTC ACC ATT ATC GTT TCA GAC CCA CCT CCC ATC CG

HVD-1 GAT CAG CAT GCC TGC AGG TCG ACT CTA GAC CCG GGT ACC GAG CTC GCC CTA TAG TGA GT C GTA TTA T  
HVD-2 CCG GAT AAT ACG ACT CAC TAT AGG GCG AGC TCG GTA CCC GGG TCT AGA GTC GAC CTG CAG GCA TGC T

## (B) Replacement of U1 sequences with HIV Anti-sense sequences

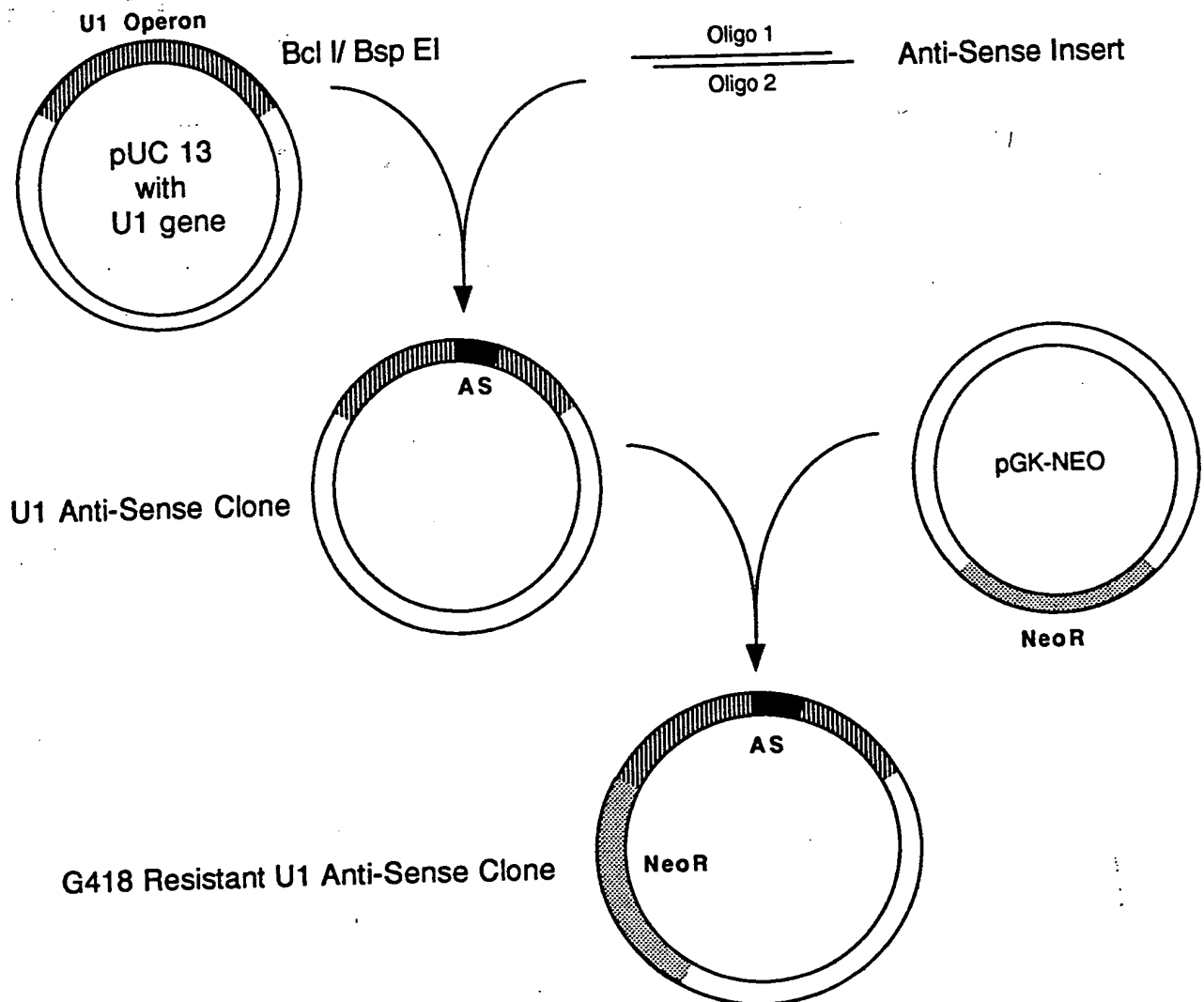
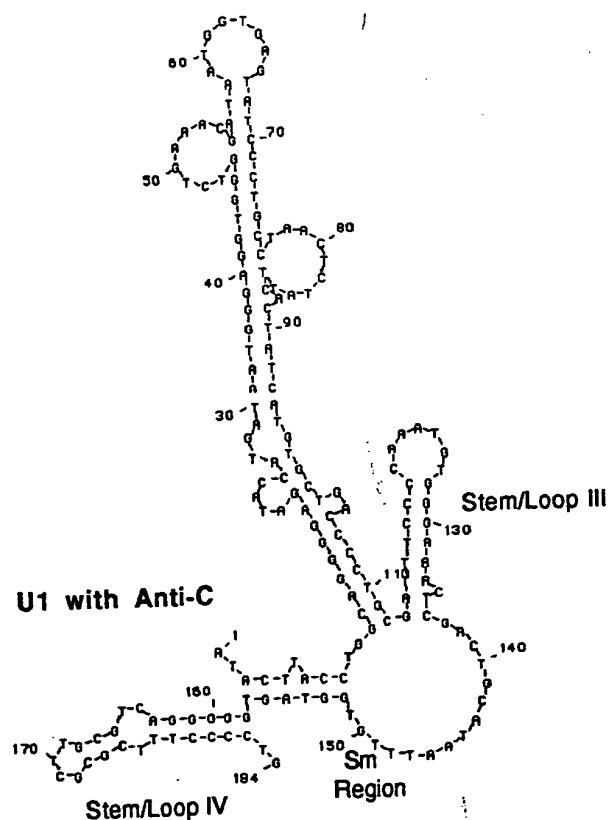
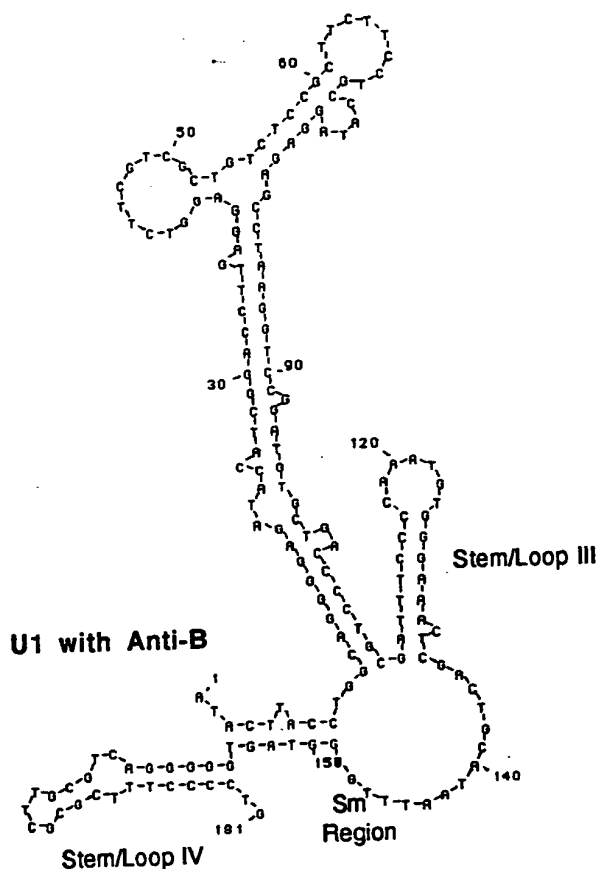
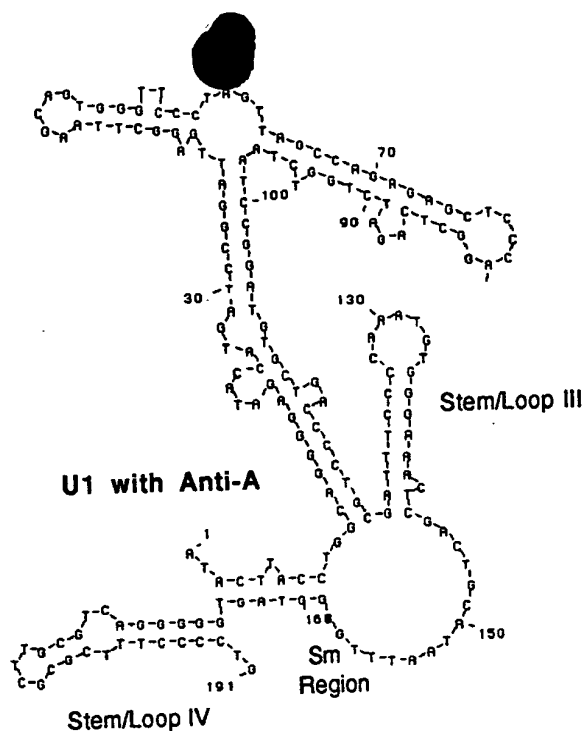
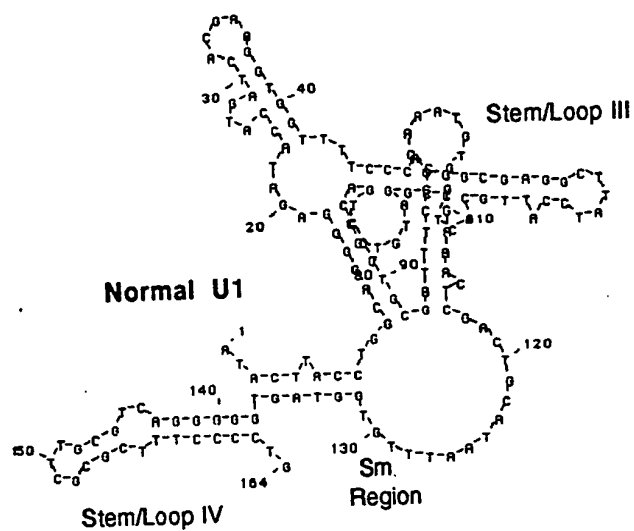


Figure 42

Insertion of Anti-Sense Sequences into U1 Operons

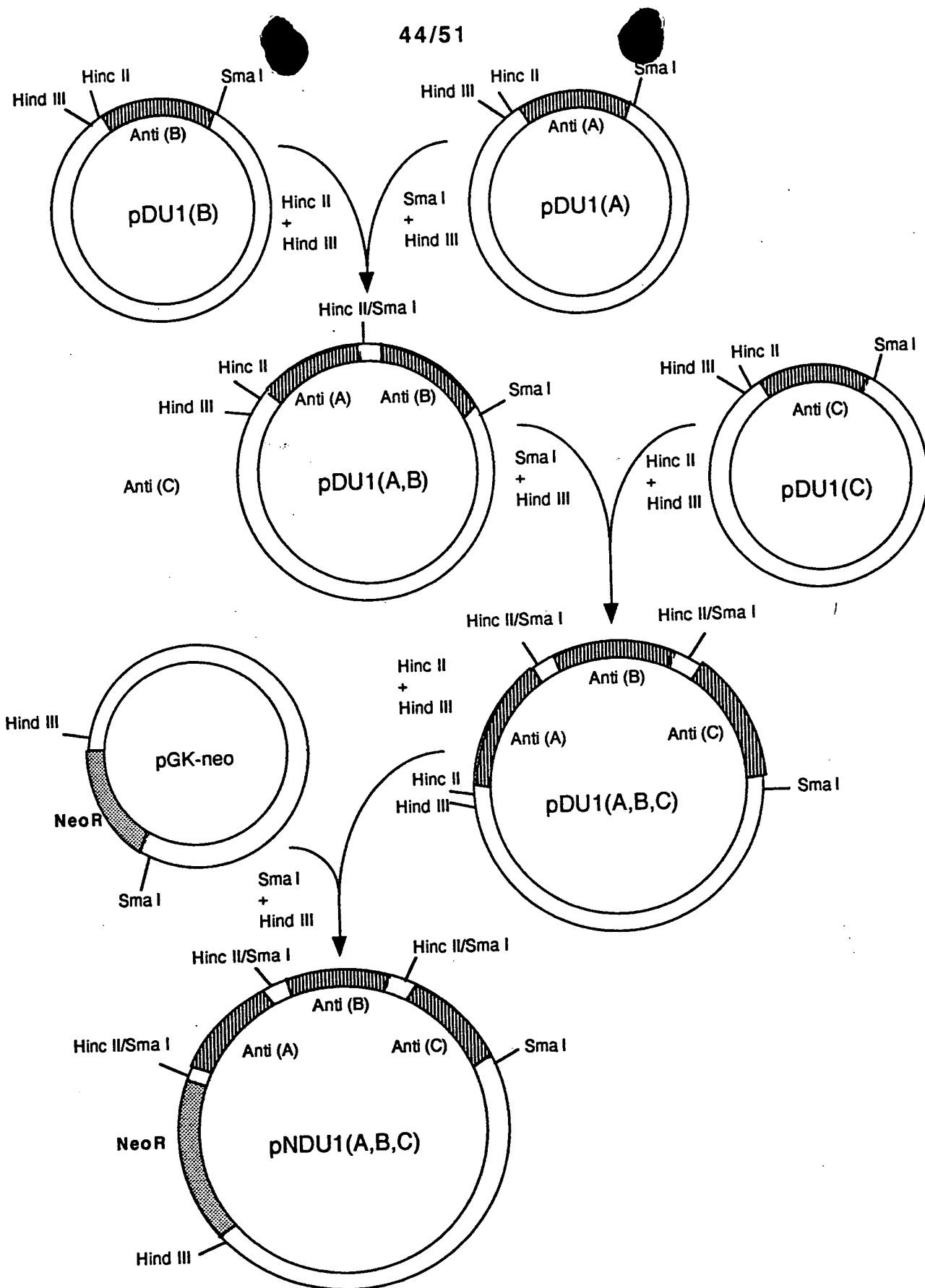


**Figure 43**

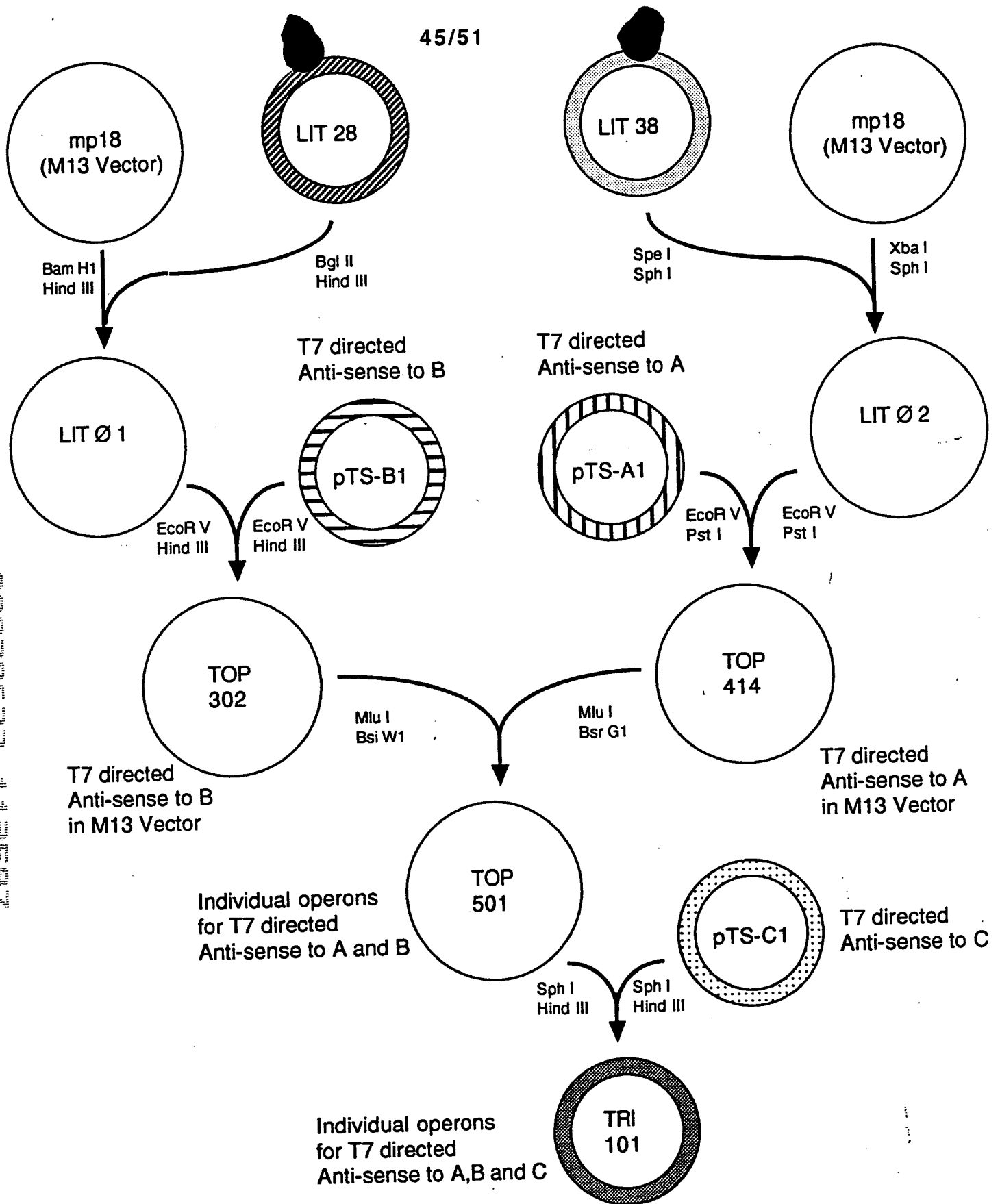
Predicted Secondary structures for U1  
Transcripts with Anti-sense Substitutions

08978632-11597

44/51



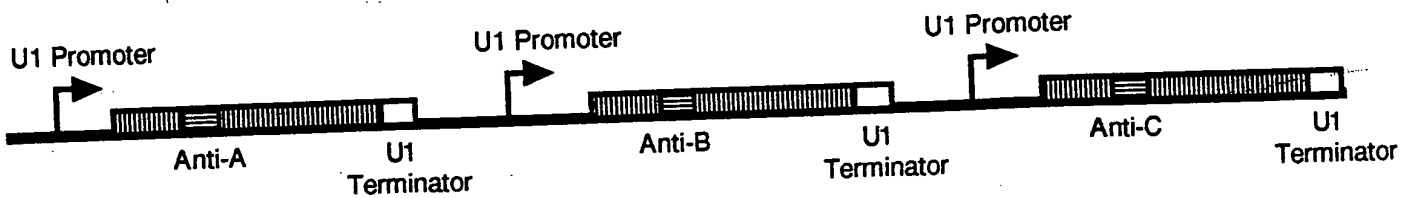
**Figure 44**  
Construction of U1 Multiple Operon Clone



**Figure 45**  
**Construction of T7 Triple Operon**

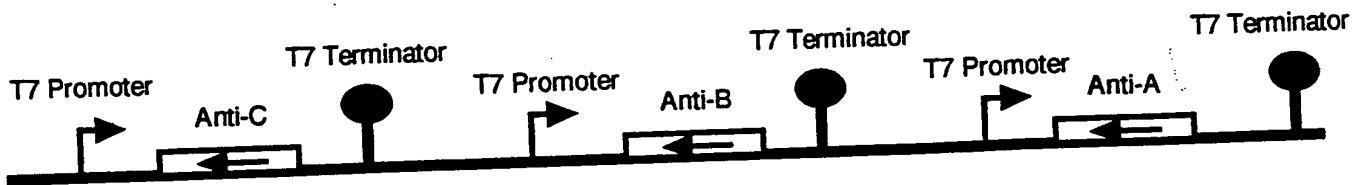
# pNDU1(A,B,C)

Triple U1 Operon Construct with HIV Anti-Sense



# TRI 101

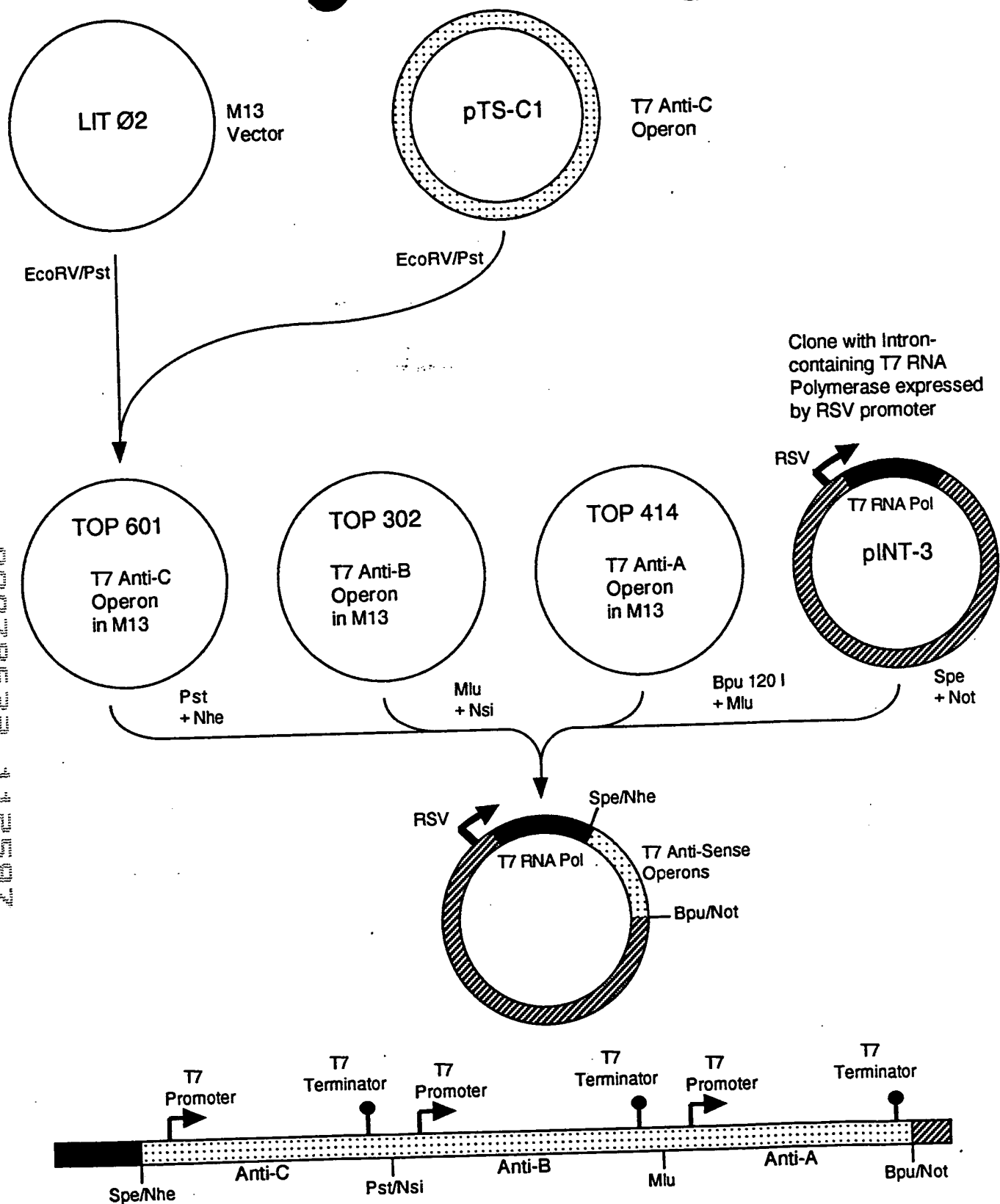
Triple T7 Operon Construct with HIV Anti-Sense



**Figure 46**

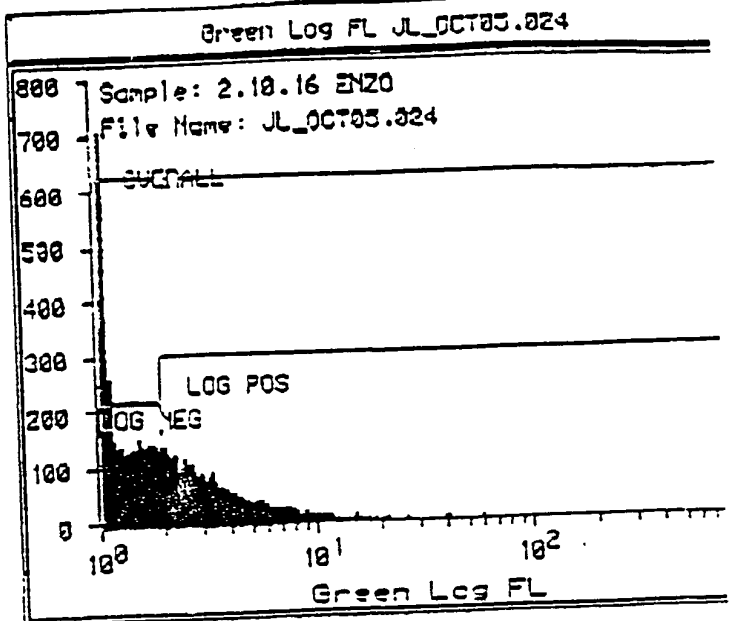
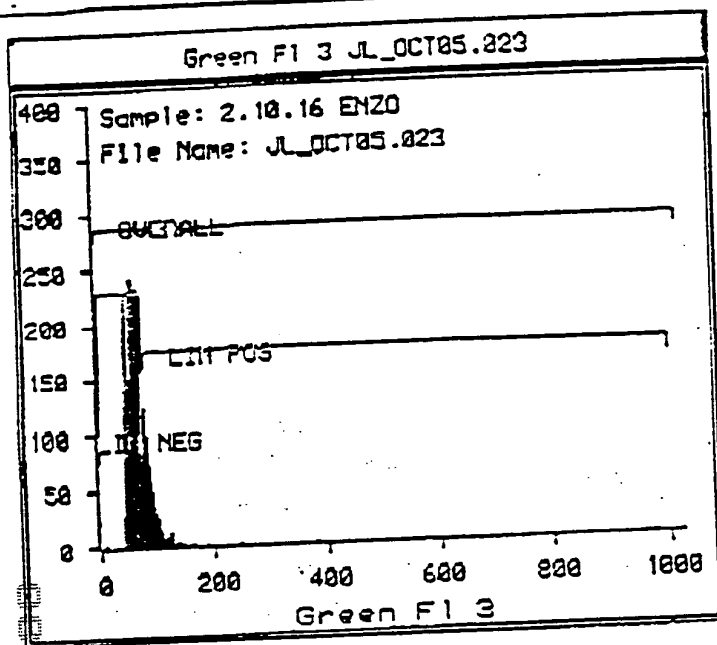
Structures of Triple Operon Constructs  
from Figures 44 and 45

00078632-112597



**Figure 47**

Construction of Multiple T7 Operons in Vector coding for T7 RNA Polymerase



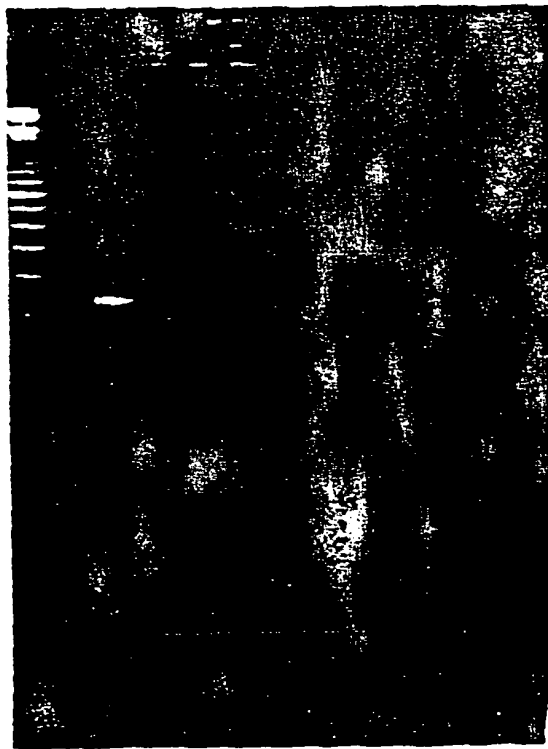
Global Statistics									
1. Green F1 3 JL_OCT05.023				Total = 7509					
2. Green Log FL JL_OCT05.024				Total = 7509					
Hist	Region	Bounds	Counts	x	Mean X	Mean Y	Mode	%	
1.	LIN NEG	1 78	5714	76.1	63.65		70	14	
	LIN POS	85 1002	1129	15.0	97.34		85	17	
	OVERALL	1 1024	7509	100.0	70.28		70	23	
2.	LOG NEG	2 2	4211	56.1	2.34		2	21	
	LOG POS	2 1001	3407	43.4	4.76		2	69	
	OVERALL	2 1001	7509	100.0	3.43		2	88	

Figure 48

Flow cytometry data measuring binding of  
anti-CD4+ antibody to HIV resistant U037 cells



+ 10-601  
 1 A-1014



PCR HIV-1 gag - A 2 clone

Figure 49

PCR amplification of gag region  
 indicating absence of HIV in  
 viral resistant cell line (2.10.16)  
 after challenge

BEST AVAILABLE COPY

08978632.113597

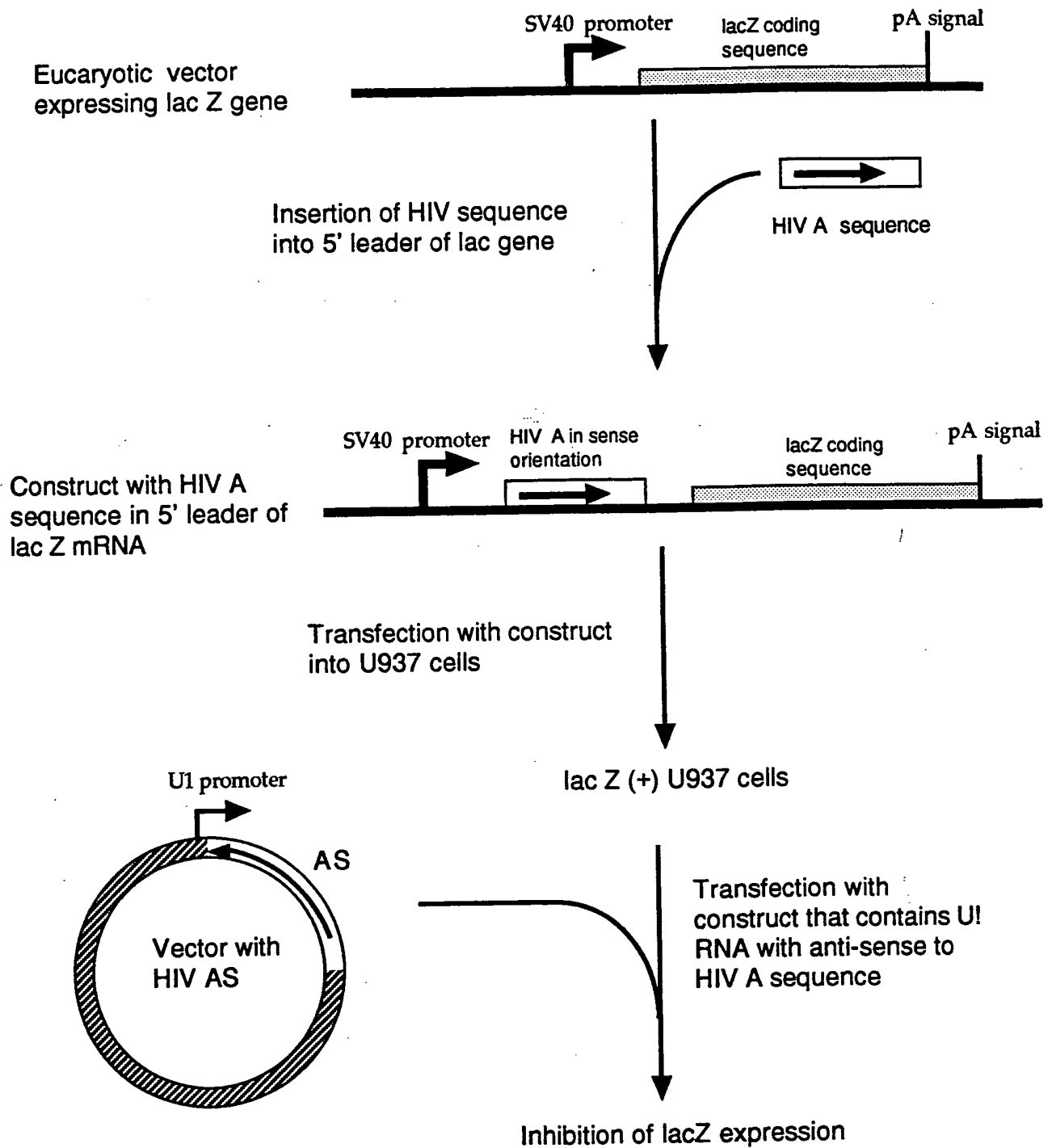


Figure 50

Clone with target-lacZ fusion will have reduced expression of lacZ after transfection by HIV Anti-sense construct

Enzyme activity as expressed by  $A_{420}$  readings  
in extracts prepared from

	$2.5 \times 10^4$ cells	$5 \times 10^4$ cells	$1.0 \times 10^5$ cells
U 937 [untransfected]	0.018	0.023	0.034
U 937 [ HIV A clone ]	0.154	0.277	0.566
U937 [ HIV A / Anti-A]	0.010	0.017	0.027
U 937 [ HIV A/Anti-ABC]	0.013	0.021	0.035
U 937 [ HIV A / Null DNA]	0.120	0.212	0.337

[ B ] Expression of Beta-galactosidase activity by *In situ* assay :

U 937 [ untransfected ]    no blue spots in cells  
 U 937 [ HIV A clone ]    blue spots in cells  
 U 937 [ HIV A/Anti A ]    no blue spots in cells  
 U 937 [ HIV A/Anti ABC]    no blue spots in cells  
 U 937 [ HIV A / Null DNA] blue spots in cells

Figure 51

Expression of Beta-galactosidase activity  
in extracts

08970632-112597  
265277-22992680